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# PROCEEDINGS

OF THE

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# SOCIETY FOR
# EXPERIMENTAL BIOLOGY AND MEDICINE

INCLUDING THE
PACIFIC COAST BRANCH, MINNESOTA BRANCH AND
WESTERN NEW YORK BRANCH.

VOLUME XIX
1921–1922

EDITED BY THE SECRETARY

NEW YORK
1922

23-90558 Jan. 31
5

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

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# SCIENTIFIC PROCEEDINGS.

## One hundred seventeenth meeting.

### Abstracts of Communications.

*Cornell University Medical College, October 19, 1921.*
*President Wallace in the chair.*

### 1 (1748)

### Further studies on the nature of botulinus toxin.

By **J. BRONFENBRENNER** and **M. J. SCHLESINGER.**

[*From the Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston, Mass.*]

In trying to duplicate in vitro the conditions as they exist when botulinus toxin is taken by the mouth, we have observed that the acidity equal to that of the stomach contents not only leaves the toxicity of botulinus toxin undiminished, but would actually increase its potency. It has been repeatedly stated in the literature that botulinus toxin resists action of acids, but so far as we know nobody has observed the increase in potency of this toxin resulting from the change in its hydrogen ion concentration.

In attempting to establish the extent of this increase in potency we found that under the suitable conditions of the experiment the botulinus toxin which ordinarily kills mice in amounts not smaller than $3 \times 10^{-7}$ cc. can be increased in potency to such an extent that $3 \times 10^{-21}$ cc. occasionally and $3 \times 10^{-18}$ cc. quite regularly kills mice of 18–20 grams in less than 48 hours after the intraperitoneal injection. While the total solids of such a minute dose of toxin amount to only $3 \times 10^{-23}$ grams (this amount including also the inorganic portion of the medium) the toxic product thus obtained, nevertheless, possesses all the essential characteristics of bacterial toxins: it is thermolabile, it acts only after an incubation period, it reproduces in experimental animals typical symptoms of the botulinus poisoning and it exhibits strict specificity in its neutralization with the homologous antitoxin.

Our studies, thus far, were limited to the toxin produced by a single strain of *Bacillus botulinus*, but the experiments are in progress to determine whether the observation can be extended to toxins produced by other strains of *Bacillus botulinus* as well as to toxins of other bacteria.

This work is a part of the investigation of food poisoning, conducted under the direction of Dr. M. J. Rosenau, professor of preventive medicine and hygiene, Medical School of Harvard University. The investigations are made under the auspices of the Advisory Committee on the Toxicity of Preserved Foods of the National Research Council, and under a grant of the National Canners' Association.

### 2 (1749)

## Some plant sources of vitamins B and C.

By **FRANCISCO O. SANTOS.**

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

Several plant foods were tested for their content of vitamins B and C. Togi (sprouted mongo), okra, and avocado were found to be comparatively high in vitamin B. One half gram of each of them as daily supplement to the standard vitamin B free diet caused the recovery in weight of rats which had been declining because of lack of this accessory food factor. Mongo, sweet potato leaves and duhat (*Eugenia jambolana*) contain enough vitamin so that one gram of each of them as daily supplement caused the recovery in weight of rats which had been declining due to lack of vitamin B. Artichokes, bilimbi (*Averrhoa carambola*), banana flower bud and bamboo shoots are relatively poor in vitamin B.

The vitamin B in mongo was increased in germination, a fact contrary to the finding of Grijns that the antiberi-beri vitamin is lessened in amount as germination takes place.

Mongo is relatively poor in vitamin C. Togi when fresh is relatively rich in vitamin C; but after it is prepared for culinary use, the vitamin C is destroyed.

The observation of several investigators that vitamin C is increased when peas, lentils, and beans are germinated has been verified in the case of mongo. Ten grams of mongo as daily supplement to the scorbutic diet failed to protect guinea pigs from scurvy, while five grams of fresh togi as supplement to the same scorbutic diet cured three guinea pigs of the disease.

### 3 (1750)

## Observations on pancreatic rennet.

By **ALBERT A. EPSTEIN.**

[*From the Department of Physiological Chemistry, Mt. Sinai Hospital, New York City.*]

Pawlow and Parastschuk,[1] Vernon[2] as well as Delezenne[3] have called attention to the presence of rennet in the pancreatic secretion of experimental animals. Wohlgemuth[4] claims to have found it in human pancreatic secretion, but not without some difficulty. Notwithstanding these observations some doubt seems to exist in the minds of a number of investigators in this field. Textbooks on physiology do not class rennet with the other pancreatic ferments.

Fresh or well-preserved dried preparations of pancreatic extract ordinarily do not show any milk coagulating ferment. When solutions of such extracts are permitted to deteriorate the rennet function comes into evidence. While studying the pancreatic ferments I have found that the presence of rennet in extracts of this organ may be demonstrated constantly in a number of different ways.

1. Rennet may be liberated by heating a solution of the extract from 50 to 65° C. for a period of about 10–15 minutes; the most favorable temperature being 60° C. Flocculation usually occurs upon heating, but the ferment remains in solution.

2. The addition of suitable amounts of hydrochloric acids reveals the presence of rennet.

---

[1] Pawlow, J. P., and Parastschuk, S. W., *Zeitschrift fur Physiologische*, 1904, xlii, 415.

[2] Vernon, H. M., *Journal of Physiology*, 1903, xxix, 302.

[3] Delezenne, *Soc. Biol.*, 1907, lxiii, 98.

[4] Wohlgemuth, *Biochem. Zeitschrift*, 1917, ii, 350.

3. By treating solutions of pancreatic extract with colloidal iron and other precipitants such as uranium acetate, alcohol, sodium sulphate and others. Calcium chloride solution in concentration accomplishes the same result.

4. The addition of products of peptic digestion, such as those of gliadin or Witte's peptone, to solutions of pancreatic extract also liberate the rennet.

5. Serum of a rabbit immunized by intravenous injections of pancreatic extracts, when added to solutions of pancreatic extract, liberate the rennet.

Whatever method of activation is used, in every instance, the rennet itself remains in solution, and some substance is precipitated, which before precipitation conceals the presence of the rennet.

I reported some of these results at a Section meeting of the American Chemical Society, held last September, and concluded at the time that the rennet in the pancreatic extract was probably present not as a pro-enzyme, but as an active enzyme mixed with substances which are antagonistic to its action. The conclusion is based on the foregoing experiments, the most significant of which is the one showing the effect of immunized serum on inactive pancreatic extract. Apparently the inactive solution of pancreatic extract is capable of producing in an immunized animal an antibody for the substance in the pancreatic extract, which is antagonistic to the rennet. The antibody thus produced is in the nature of a precipitin. The lack of any specific method of activation seemed to support this view that there is no pro-ferment. However, the proof is indirect, hence not final. Various attempts to recover the antagonistic substance in active form proved futile. The most that can be said about it is that it probably is a substance of protein nature which coagulates at a temperature between 60 and 65° C., is precipitable by sodium sulphate and other precipitants, and is capable of producing a precipitin in immunized serum.

These facts brought to mind the former controversy concerning the nature or state of the rennet in gastric mucosa.

You will recall that rennet is believed to exist in two states, that of an active enzyme (or rennet) and as a pro-enzyme (or pro-rennet), and that under the influence of very small quantities of acid at the optimum temperature, the pro-rennet is rapidly transformed into the active rennet. This result is regarded as the product of true activation. Hedin[5], however, interprets the facts in another manner. He assumes that the pro-rennet is merely a combination of rennet with a substance antagonistic to it, and on the following grounds. If the pro-rennet be treated with dilute HCl, the rennet is set free and the antagonistic substance destroyed, hence, its inhibitory action is lost. On the contrary, a solution of pro-rennet, treated with very dilute ammonia at 37° C., loses all its rennet already free, while the antagonistic substance remains unchanged; so that, by adding active rennet to this treated liquor, the rennet is at once rendered inactive.

I applied Hedin's method of proof to the rennet in the pancreatic extract and found that the results were in accord with his. On closer analysis it became evident that Hedin's proof was insufficient and the conclusion erroneous.

Before proceeding to the evidence in substantiation of this, permit me to note the following concerning the pancreatic rennet. The content of this enzyme in pancreatic extract is very large, and goes absolutely hand in hand with the quantity of trypsin present. Means have not yet been found to separate rennet from trypsin. The two appear to be intimately associated functionally and chemically. A method has been devised for the quantitative recovery of the rennet-trypsin enzyme and for its purification. This will be presented at another time. Suffice it to say for the present that the trypsin-rennet combination constitutes about 1–2 per cent. of the total substance of dried pancreatic extract. It is of protein nature, is not precipitated by colloidal iron, is coagulated by heat (at 82–85° C.), is extremely hygroscopic, and of an acid character.

It is active only in the presence of Ca, which, however, must be available in ionizable form. In this fact seems to lie the fault in Hedin's proof.

I have stated that fresh or well-preserved pancreatic extract has no milk coagulating properties, but when a solution of it is treated by heating (up to 60° C.) or by means of colloidal iron and other precipitants, the rennet is set free. Now if some of the original extract is added to the activated preparation, no inhibition of the rennet action occurs. In other words, none of the substance which hinders the action of rennet is present in a free state in the pancreatic extract. When alkalinized with ammonia the original extract acquires the power to inhibit the action of active rennet preparations. The result, however, is not due to the setting free of the antagonistic substance (as Hedin believes), but to the fact that the calcium ion, which is essential for coagulation, is rendered inert by the procedure. On the other hand, if a solution of active pancreatic rennet is alkalinized with the hydrate or carbonate of ammonia or soda, the enzyme solution is rendered inactive. If, however, $CaCl_2$ in sufficient amounts is added to this liquor, the rennet is immediately reactivated. Neutralization or alkalinization of the active rennet solution by means of disodic phosphate, or calcium hydrate, does not inactivate the rennet.

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[5] Hedin, S. G., Harvey Lectures, 1914, p. 162.

It would appear from this that Hedin's result was not due to inactivation of the rennet by means of the antagonistic substance, but merely to the removal of the calcium ion from the sphere of action.

The experiments made thus far seem to indicate that the enzyme substance forms a chemical combination with calcium, in the nature of a salt, and only as such exerts its action. There appears to be some ground for the belief that rennet and trypsin reside in a single chemical unit of the pancreatic substance, and possibly represent two phases of one and the same ferment.

I might add in conclusion that, whereas the evidence is in favor of the view that the rennet in pancreatic substance exists as an active enzyme and not as a pro-rennet, definite proof for this opinion is still lacking.

### 4 (1751)

## Further observations on the seat of the emetic action of the digitalis bodies.

By ROBERT A. HATCHER and SOMA WEISS.

[*From the Department of Pharmacology, Cornell University Medical College, New York City.*]

The application of digitalis bodies directly to the vomiting center, described by Thumas, does not cause emesis.

When a digitalis body is injected into a cat in which the spinal cord has been cut at about the level of the second thoracic vertebra, vomiting does not usually occur, but when the cord is severed at the level of the 5th thoracic vertebra vomiting is not prevented.

Removal of the stellate ganglia frequently prevents this emesis, and removal of the stellate ganglia with cutting of both vagi prevents the emesis in nearly every case.

Removal of the celiac plexuses does not interfere with the emesis following the injection of digitalis bodies.

When the nerve supply to the heart is intact the injection of a digitalis body causes emesis, if the animal is in good condition. When all nerve supply to the heart is severed, digitalis does not cause emesis, but mercuric chloride still causes vomiting in the usual way.

Impulses appear to pass up from the heart to the vomiting center chiefly by the way of the sympathetic, and to a less, though probably variable, extent by way of the vagus. When the sympathetic is cut the administration of ouabain *usually* fails to induce emesis. This may be due to the fact that the impulses passing up the vagus are usually insufficient to set up the coördinated reflex, or it may be that in those cases where vomiting is not elicited by this drug after the sympathetic is cut the vagus does not contain any fibers concerned in this reflex.

Evidence is presented to show that digitalis bodies induce emesis by reflex action due to irritation of the heart or its appendages.

This is almost certainly a protective mechanism for the heart such as is recognized in the case of other organs.

We wish to offer the *suggestion* that impulses constantly pass from various organs to the vomiting center and that apomorphin promotes the coördinated vomiting reflex to such a degree that these normal impulses give rise to vomiting.

That the action of apomorphin on the vomiting center is strictly analogous to that of strychnin on the cord whereby convulsions—apparently spontaneous, but in reality of reflex character—are induced.

Vomiting requires powerful—almost *convulsive*—contractions of the abdominal muscles and diaphragm, and the weak stimuli are incapable of setting up the reflex in the unpoisoned animal.

It is significant, too, that morphin produces strychnin-like convulsions through its action on the cord (in the frog), and apomorphin-like emesis through its action on the medulla.

### 5 (1752)

## III. Experimental rickets.

## The prevention of rickets in rats by exposure to sunlight.[1]

By **ALFRED F. HESS, L. J. UNGER** and **A. W. PAPPENHEIMER.**

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

In recent papers it was shown by Hess and Unger that rickets in infants could be cured by frequent short exposures to the sun's rays.[1, 2] By this means and without any alteration whatsoever of the dietary, the characteristic signs of this disorder begin to disappear in three to four weeks as noted by clinical examination and by the x-ray. As a result of favorable experiences of this nature, it was concluded in a study of the seasonal incidence of rickets[3] that "hygienic factors, especially sunlight, and not dietetic factors play the dominant rôle in the marked seasonal variations of this disorder." It seems probable that the ultra-violet rays play a large part in this curative power of the sun, judging from the work of Huldschinsky[4] and others who recently have shown that infantile rickets can be cured by means of the rays produced by the mercury-vapor lamp. In 1918 we tried the curative effect of rays from this source, but, lacking the aid of x-ray examinations, could not convince ourselves of their efficacy; since then we have succeeded in curing rickets by this means.

---

[1] Hess, A. F., and Unger, L. J., PROC. SOC. EXPER. BIOL. AND MED., 1921, xviii, 298.

[2] Hess, A. F., and Unger, L. J., *J. A. M. A.*, 1921, lxxvii.

[3] Hess, A. F., and Unger, L. J., *Amer. J. Dis. Child.*, 1921, xxii, 186.

[4] Huldschinsky, K., *Zeitschr. f. orthop. Chir.*, 1920, xxxix.

Having found sunlight efficacious in the rickets of infants, we proceeded to test its value in the prevention of rickets in rats. To this end a series of white rats were placed on the diet (No. 84) described by Sherman and Pappenheimer,[5] consisting of patent flour 95.0 per cent., calcium lactate 2.97 per cent., sodium chloride 2.0 per cent. and ferric citrate 0.1 per cent. It has been the experience of the investigators in this laboratory that such a diet invariably leads to the development in rats of lesions which are anatomically identical with those of infantile rickets.

In carrying out experiments on rats our practice has been to keep the colony in a semi-dark room, the yellow shades being drawn at all times. In testing the effect of sunlight, the rats (weighing at the outset about 40 grams) were kept in absolute darkness, one series being taken out of the room and exposed to the direct sunlight for a period of 15 or 30 minutes. There was no difference whatsoever in the diets of these two groups. After a period of about three weeks the animals were x-rayed in order to observe early lesions of the epiphyses, and after thirty to forty days were killed and autopsied. These experiments were begun in April when the weather permitted four to five exposures a week.

It was found for the first time in our experience that diet No. 84, the "rachitic dietary," did not lead to rickets—that the rats which received sun treatment did not show signs of rickets either by x-ray or by histological examination of the bones. It is unnecessary to discuss in detail the histological criteria which we consider characteristic of rickets, as this question has been fully considered in a previous paper.[6] It may be stated briefly that they consist of increased width and irregularity of the proliferative cartilage, absence of calcium deposition and great excess of osteoid in the region of the metaphysis and along the shafts of the bones.

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[5] Sherman, H. C., and Pappenheimer, A. M., *J. Exper. Med.*, 1921, xxxiv, 189.

[6] Hess, A. F., McCann, G. F., and Pappenheimer, A. M., *J. Biol. Chem.*, 1921, xlvii, 395.

In the paper previously referred to it was shown that the introduction of 0.4 per cent. secondary potassium phosphate ($K_2HPO_4$) in place of an equal weight (replacing about one seventh) of the calcium lactate contained in the rickets-producing diet, completely prevented the development of rachitic lesions; this constitutes an addition of 75 mg. of phosphorus per 100 gm. of the diet. In order to test the counterbalancing effect of phosphate and darkness, a series of tests were carried out in the dark with additions of small and increasing amounts of potassium phosphate to the standard dietary (No. 84); to one series 25 mg. were added, to another 75 mg. (constituting dietary No. 85).

The rats on these diets were kept in the dark but, to serve as control, half of each series were exposed to sunlight for thirty minutes daily when this was possible. As was to be expected in view of our previous experience and the fact that phosphate tends to protect against rickets, none of the rats which were treated with sunlight developed rachitic lesions. Among the group, however, which were kept at all times in the dark, active rickets developed in spite of an addition of 25 mg. of phosphorus. The addition of 75 mg. was found to be sufficient to prevent the development of this disorder in some of the rats. This amount contituted somewhat less than the minimum protective supplement to diet No. 84, which in itself contains about 86 mg. of phosphorus. Thus it will be noted that a short exposure to sunlight was equivalent to almost doubling the protective dose of phosphate. If the phosphate content of the diet is adequate, rats do not develop rickets in spite of being kept in the dark throughout the experiment. The effect of sunlight with other dietaries was also studied, and is being continued.

## DISCUSSION.

As sunlight has a marked effect on the bony development of rats, it is evident that in future in similar nutritional investigations, the light factor will have to be controlled and standardized. It seems probable that some of the irregularities and lack of conformity observed by investigators in this field may be attributed to keeping the experimental animals under dissimilar intensities of

A. DARKNESS.

| Diet.[1] | Duration (Days). | Rat No. | X-Ray. | Microscopic Examination. |
|---|---|---|---|---|
| No. 84 | 34 | 246 | R. | R. |
| | 23 | 247 | — | R. |
| | 22 | 248 | — | R. |
| | — | 436 | R. | — |
| | — | 437 | R. | — |
| | 30 | 438 | R. | R. |
| No. 84 + 25 mg. P. | 39 | 262 | R. | R. |
| | 39 | 263 | R. | R. |
| | 39 | 264 | R. | R. |
| | 28 | 443 | R. | — |
| | 28 | 444 | R. | R. (slight) |
| | 28 | 445 | R. | — |
| No. 84 + 75 mg. P. | 38 | 121 | neg. | neg. |
| | 38 | 122 | neg. | neg. |
| | 38 | 123 | neg. | neg. |

B. SUNLIGHT.

| Diet. | Duration (Days). | Rat No. | X-Ray. | Microscopic Examination. |
|---|---|---|---|---|
| No. 84 | 34 | 249 | neg. | neg. |
| | 32 | 250 | " | " |
| | 35 | 251 | " | " |
| | 33 | 439 | " | " |
| | 33 | 440 | " | " |
| | 33 | 441 | " | " |
| | 33 | 442 | " | " |
| No. 84 + 25 mg. P. | 39 | 259 | " | " |
| | 39 | 260 | " | " |
| | 39 | 261 | " | " |
| No. 84 + 75 mg. P. | 38 | 124 | " | " |
| | 38 | 125 | " | " |

R. = rickets.

light. The most interesting aspect of the question, however, is the phenomenon that the sun's rays are able to stimulate a deposition of inorganic salts where these are lacking. The damaging effect of darkness emphasizes the fact that sunlight is of great importance, not merely for the vegetable world but also for the higher animals. Furthermore, the fact that sunlight is efficacious in the rickets of both human beings and rats, serves to show the similarity of this disorder in these two species. These results indicate that in the prevention and causation of rickets at least one hygienic factor plays an important rôle which will have to be carefully considered in future studies of this disorder.

---

[1] Diet No. 84 as originally constituted, contained 86 mg. per cent. of phosphorus. In the fall, however, owing to a variation in the phosphorus content of the flour, this diet was found by analysis to contain only 72 mg. per cent. Rats numbered our 400 (in this table) were fed on the ration having the lower phosphorus content.

### 6 (1753)

## Identical twins in pigeons arise from ova of markedly aberrant size.

By **OSCAR RIDDLE.**

[*From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.*]

During 10 years data have been accumulated for yolk size and total egg size in 15,000 to 18,000 eggs of doves and pigeons. Such measurements of these two associated structures permit us, within certain limits, to know some definite things concerning the size of either structure if the weight of the other is known. Another group of 15,000 to 20,000 eggs have been weighed, incubated, and later observations made upon the embryos and young. Incidental to these latter observations 7 instances of identical twins have been found. Such twins other than the seven listed here have almost certainly not appeared; or, if present, they attained a stage of less than 2-day embryos.

The figures of Table I make it clear that at least most of the particular eggs which gave rise to twins were of markedly different size from all other eggs then being produced. This is particularly well shown in the first four instances—given in the upper eight rows of figures—since the twin-bearing egg was in these four cases by far the *largest* egg produced by its parent during one entire year,—and so much larger as to indicate, in all probability, that it contained the largest ovum produced during the year. The seventh case was likewise of aberrant size—being the *smallest* of a group of undersized eggs. However, the weights of all eggs obtained in connection with this seventh case, as also with cases 5 and 6, are known to be rather unreliable indices of the weights of the enclosed yolks because the parent birds (K469, P843) suffered from special reproductive disorders which involved the production of irregular and inadequate quantities of shell and albumen, unpaired eggs and embryos often incapable of hatching. The early death of these three pairs of twins is probably to be associated with this circumstance. The significance of the egg weights of the four cases listed at the top of the table is wholly clear since differences of 15 per cent. in egg size (between the two eggs of a clutch) have been found in normal birds to reliably indicate that differences of yolk size lie in the same direction. The two cases listed at the top of the table have been earlier fully described[1] and the data given there will likewise demonstrate the abnormally large yolk size which must have been present in cases 3 and 4 of the present tabulation.

Apparently the known facts concerning these cases of twins do not well accord with a strict application of Stockard's[2] conclusions as to the cause of twinning and "double monsters," particularly as he has described it in relation to birds, since in the present cases we learn that the twin-producing ovum of the pigeon is "marked" for twinning even before it leaves the ovary. However, it seems possible that even these instances may fall within the range of his general explanation. We have learned that extraordinary yolk-size means a low oxidizing level of the ovum. Since this level is lowest in the largest ovum of the given bird this exceptionally low level may account for the first 4 cases of the list. In the last three cases the size of the contained ova is questionable but in these cases a disorder of the reproductive organs—already known to involve the abnormal functioning of some of the endocrine glands—may conceivably effect a retardation of development previous to gastrulation as Stockard's theory demands. The early death of many of these particular embryos, as well as an apparent excess of twins derived from the meager amount of this material, may afford evidence for such retardation. It should also be stated that in case 3 the embryo was subjected to ice-box temperatures (13°–16° C.) during the first 23 hours after laying; and that the parent in case 4 was a generic hybrid.

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[1] Riddle, O., *Jour. Exp. Zoöl.*, 1918, xxvi, 227.

[2] Stockard, C. R., *Amer. Jour. Anat.*, 1921, xxviii, 115.

The sex of the twins from the large twin-yielding ova (sex known for first 3 cases only) is of real importance; for, much earlier work by the author has shown that in pigeons the females arise from larger ova and males from smaller ova. Each of the three present cases supplies a rigorous test of the validity of that conclusion,—and each affords a confirmation. If either of these extraordinarily large ova had produced *male* twins, it would have directly contradicted the conclusions drawn from related lines of study conducted over a period of several years. If prospectively male twins were present in this series they were necessarily confined to origins from yolks of relatively small size.

TABLE I.

SIZE OF EGGS YIELDING TWINS COMPARED WITH OTHER EGGS FROM SAME BIRD.

| No. of Female Parent. | Clutch. | Data on Eggs of Twin-bearing Clutch. | | | | Average Weight of 5 Eggs Laid by Same Female Immediately: | | Maximum and Minimum Weights for Other Than Twin-bearing Eggs (Same Year). | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Date. | W't. | Per Cent. of Diff. | Sex (or Stage). | Before.[1] | After.[1] | Maximum. | Minimum. | Total |
| A248... | K1 | 4/7 | 7.43 | | ♂ | 7.62 | 8.11 | 8.22 | 7.10 | 23 |
| | K2 | 4/9 | *10.63* | +43.1 | ♀ ♀ | 8.75 | 8.92 | 9.17 | 8.46 | 22 |
| 60... | F1 | 3/5 | 8.07 | | ♂ | 8.21 | 7.58 | 8.68 | 6.72 | 22 |
| | F2 | 3/7 | *10.08* | +24.9 | ♀ ♀ | 8.13 | 7.84 | 8.65 | 6.15 | 21 |
| V49[2] .. | D1 | 10/16 | 15.77[2] | | ♂ | 14.80 | 17.05 | 17.40 | 14.80 | 8 |
| | D2 | 10/18 | *20.60* | +30.6 | ♀ ♀ | 15.33 | 18.06 | 18.23 | 15.33 | 7 |
| P450... | D1 | 5/30 | 8.92 | | ♂ | 8.76 | 9.02 | 9.21 | 7.82 | 17 |
| | D2 | 6/1 | *10.39* | +16.5 | (4–5d.) | 8.90 | 9.17 | 9.75 | 8.12 | 14 |
| K465... | E1 | 3/4 | 8.00 | | (1.0d.) | 7.59 | 7.95 | 8.87 | 6.87 | 17 |
| | E2 | 3/6 | *8.05* | + 0.6 | (2.5d.) | 8.24 | 8.50 | 9.29 | 7.82 | 13 |
| K465... | K | 5/14 | *8.47* | —— | (4.5d.) | 7.95 | 8.18 | 8.87 | 6.87 | 17 |
| | | | | | | 8.50 | 8.64 | 9.29 | 7.82 | 13 |
| P843... | C1 | 3/15 | *6.40* | − 3.7 | (3.0d.) | 7.51 | 7.04 | (?) | (?) | 2 |
| | C2 | 3/17 | 6.64 | | (2.0d.) | 7.10 | 6.90 | (?) | (?) | 3 |

[1] In some instances the twin-bearing clutch was preceded or followed by fewer than 5 eggs (see second and last columns).

[2] Common pigeon; all other groups are ring-doves.

*Note.*—The weights of twin-bearing eggs are set in italic type. First and second eggs of the clutch are kept separate throughout the table.

### 7 (1754)

## The vitamines of yeast and their rôle in animal nutrition.

By **CASIMIR FUNK** and **HARRY E. DUBIN.**

[*From the Research Laboratory of H. A. Metz, New York City.*]

The question whether pigeons and rats require for their well-being the same vitamine B has been discussed at length by Mitchell and Emmett a few years ago with the conclusion that vitamine B must be different from the antineuritic substance. Funk and Macallum have tested the phosphotungstate precipitate obtained from yeast and have found that while it was strongly curative for avian beriberi, it induced only moderate growth in rats. The present writers were able to show recently that by fractional adsorption with fuller's earth or norit it is possible in most cases to effect an almost quantitative separation of the B-vitamine, curative for avian beriberi from another substance, which we provisionally have called vitamine D and which acts on yeast and certain bacteria. In practice the separation is effected as follows: One liter of autolyzed yeast is shaken with 50 g. of fuller's earth; the filtrate which in the majority of cases was found inactive for avian beriberi was treated twice with the double amount of fuller's earth, the combined precipitates carrying down quantitatively the vitamine D, the last filtrate being devoid of the two above-mentioned substances.

Having succeeded in this separation (the procedure varying somewhat with different samples of autolyzed yeast) we thought it worth while to test out the fractions obtained on animals, making simultanous tests on pigeons, rats, yeast and streptococci. The experiments carried out with six rats and four pigeons in every case will be repeated and extended and the present communication is only of a preliminary character. While the pigeons were found to need only the vitamine B when fed on a vitamine-free diet, the rats exhibited a somewhat different behavior. They were fed the usual so-called synthetic diet with cod liver oil as source of vitamine A. The rats receiving the vitamine B or D fraction as an addition grew only for a few weeks at a slow rate and started to die out after two months. While increasing the amount of one vitamine did not have any effect, the addition of the missing component in both of the above cases caused a prompt resumption of growth. The rats given both B and D from the start together with the last filtrate, which contains neither B nor D, showed a normal behavior both in regard to growth and appearance. The influence of the last filtrate does not seem to be very important but has to be investigated.

The results suggest that the rats and possibly other mammals require, besides the vitamine A, at least two vitamines of the B type, namely the B and D vitamine, for their well-being and growth.

### 8 (1755)

## Comparative buffering value of American peptones.

By J. BRONFENBRENNER, G. G. DE BORD and P. F. ORR.

[*From the Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston, Mass.*]

Some time ago one of us[1] reported before this Society the results of the inquiry into the effect of the composition of the medium as affecting the reliability of the cultural methods of identification of bacteria, and has particularly insisted on the rôle of the buffer and on necessity of quantitative adjustment of media in respect to its buffer content.

In the present investigation we have attempted to determine the buffer content of a few of the commercial peptones with the view of determining the limits of possible variation in the buffer content in the media prepared in different laboratories as due to the choice of peptone alone. The method used was that of determining electrometrically the hydrogen ion concentration of the various peptone solutions before and after the addition to them of measured amounts of acid and alkali respectively. The study demonstrated the fact that initial reaction of different peptones varies within fairly broad limits, that due to complexity of composition the buffering action of any given peptone varies at different zones of hydrogen ion concentration, and that buffering action of one peptone at a given hydrogen ion concentration may exhibit as much as five times more buffering action than another peptone at the same hydrogen ion concentration. In general, the peptones tested showed the highest degree of variation in buffering effect in the zone of the hydrogen ion concentration limited between $P_H = 9$ and $P_H = 8$, and the lowest degree of variation in the zone between $P_H = 4$ and $P_H = 5$. As to the absolute concentration of buffering salts, these were found in most peptones to be the highest at the zone of the lowest concentration of the hydrogen ions and not in the zone of neutrality or of high hydrogen ion concentration where the buffering action would be most desirable for the use in media for identification of bacteria.

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[1] Bronfenbrenner and Schlesinger, PROC. SOC. EXP. BIOL. AND MED., 1918, xvi, 44.

Below is a table showing the relative buffering action of peptones at various $P_H$ levels.

| Peptone. | $P_H$ 9–8. | $P_H$ 8–7. | $P_H$ 7–6. | $P_H$ 6–5. | $P_H$ 5–4. |
|---|---|---|---|---|---|
| Difco | 9 | 5 | 3.5 | 5 | 11 |
| Proteose | 11 | 8 | 4 | 5 | 15 |
| Witte | 6 | 6 | 5 | 4.5 | 10 |
| Aminoid | 34 | 11 | 7 | 6 | 14 |
| Fairchild | 12 | 8 | 9 | 7 | 14 |
| Roche | 13 | 8 | 5 | 4 | 10 |
| Armour | 20 | 11 | 9 | 7 | 12 |

This work is a part of the investigation of food poisoning, conducted under the direction of Dr. M. J. Rosenau, Professor of Preventive Medicine and Hygiene, Medical School of Harvard University. The investigations are made under the auspices of the Advisory Committee on the Toxicity of Preserved Foods of the National Research Council, and under a grant of the National Canners' Association.

### 9 (1756)

## Some mathematical relations in the Wassermann reaction.

By **STERNE MORSE.**

[*From the Psychiatric Institute, Ward's Island, New York City.*]

Von Krogh's[1] equation, $y = x^n/(x^n + k)$, has not received the the consideration by immunologists which its very close statement of the facts in several immune mechanisms capable of numerical expression would warrant. It will in general, for instance, closely state the amount of hemolysis in a system where complement is the only independent variable, $x$, $y$, is the proportion of hemolysis read colorimetrically, and $n$ and $k$ are constants. It is often more convenient to use it in the form of $x^n = k[y/(1 - y)]$. If this expression is put into logarithmic form,

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[1] Von Krogh, *Journal of Infectious Diseases*, 1916, xix, 452.

$$n \log x = \log k + \log \frac{y}{1 - y},$$

the expression is linear when expressed graphically, that is, if plotted on logarithmic paper, the data will fall more or less accurately on a straight line whose slope numerically expressed will equal $n$, and whose intercept on the axis $y$ will be the reciprocal of $k$. Moreover, if two complements are compared, the intercept of their graphs on the axis of $x$ will be reciprocals of their concentration referred to any unit in which we may choose to express such concentrations.

In theory and this is to a large extent borne out in practice, this intercept on the axis of $x$ is independent of the value of $n$.

$n$ varies in the case of blood cells with the individual from which the blood is drawn, with the age of the blood cells, and with the treatment which they have experienced. It is low when the cells are suspended in Ringer's solution, high when they are suspended in salt solution, is increased with the age of cells and in general with harmful conditions, such as the presence of antiseptics in small concentration and the like. It decreases as the concentration of cells is increased. It varies under the conditions and

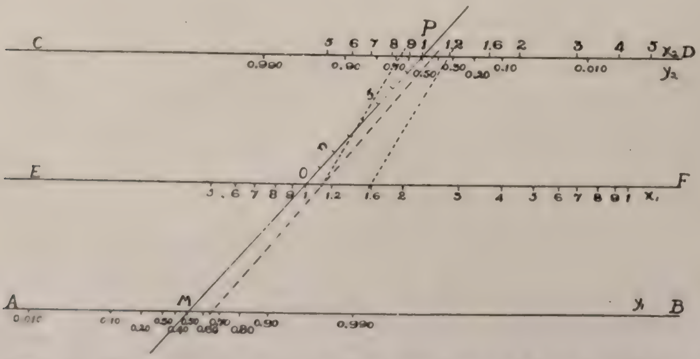

FIG. 1. Nomogram for solution of the equation $y = x^n = x^n + k$, for $n$.

technic used in this laboratory[1], from 2 to 6, generally around 3.5.

In order to calculate the constants of the above equation from numerical data, I have devised the nomogram illustrated in Fig. 1. This enables the value of $n$ to be directly ascertained from any pair of values of $x$ and $y$, $(x_1, y_1,)$ and $(x_2, y_2)$, where as before $x$ is

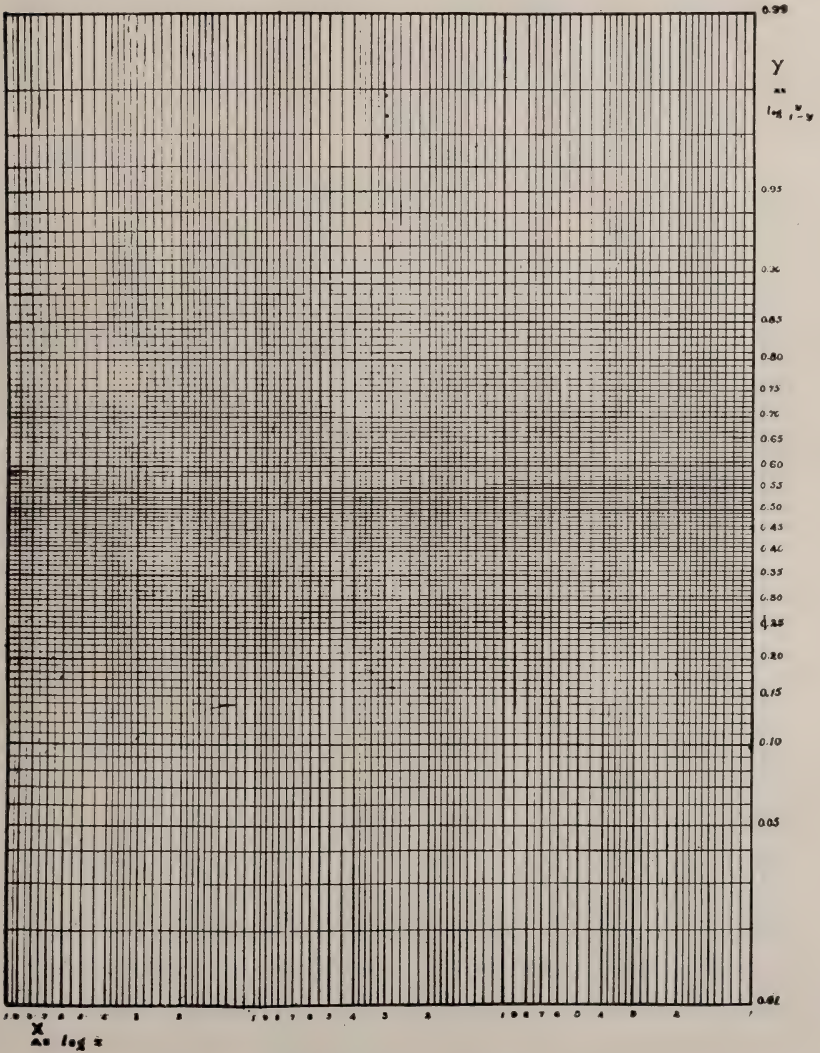

FIG. 2. Coördinate paper — log $y/(1 - y)$ *vs.* log $x$.

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[1] Morse, *Psychiatric Bulletin*, 1916, i, 47.

the independent variable, complement, and $y$ is the proportion of cells which are hemolyzed. Possibly a more convenient method still is the use of the coördinate paper shown in Fig. 2, where log $[y/(1 - y)]$ is directly compared with log $x$. In the case of the nomogram a straight edge is laid between the values of $y_1$ and $y_2$ on the lines $AB$ and $CD$ respectively and the point where it crosses the line $EF$ marked. A line is then drawn between the values of $x_1$ and $x_2$ on $EF$ and $CD$ respectively and this line is moved parallel to itself until it passes through the point where the first line crosses $EF$. It will then intersect the line $OP$ at the value of $n$ required to satisfy this pair of values. In the use of the coördinate paper the values are plotted directly and the slope measured. This second method has the advantage over the first of being dependent on all values of $x$ and $y$ and not merely upon a pair of values.

The Wassermann reaction is in the last analysis an estimation of complement after certain procedures are performed. This method is, it is believed, the nearest to an absolute measure of complement which has yet been devised and has a precision under favorable conditions of 3 per cent. or better.

The reaction between syphilitic antibody-antigen complex and complement appears to follow the same law, wherein the logarithm of the proportion between the amount of complement absorbed to that unabsorbed varies linearly with the logarithm of the amount of antigen antibody complex present, the slope of the graph in this case ranging round 1 or a little higher.

If these considerations are valid, one can make certain statements as to the Wassermann procedure which are at variance with the theory of the reaction as ordinarily conceived. In the first place, the estimation of complement should be performed under such conditions as to bring the amount of hemolysis in the neighborhood of 50 per cent., which corresponds to the value log $[y/(1 - y)] = 0$. The precision of measurement of complement by using this point can be calculated to be and is in fact at least 10 times as great as the precision obtainable by the common methods. In the second place, a true measure of the amount of syphilitic antibody antigen complex is given, not by the absolute amount of complement absorbed, but by the proportion which the amount absorbed bears to that unabsorbed. Thirdly, it follows from the second conclusion that the actual amount of complement which is used in the reaction is not important within limits, except as it affects the slope of the plotted logarithmic curve. This gives a method susceptible of considerable accuracy for the comparison of any unknown syphilitic serum or spinal fluid with a standard syphilitic serum which has previously[1] been shown to be indefinitely preservable by appropriate technic.

### 10 (1757)

## Experiment in new method of therapy of paralysis agitans.

By **M. H. WEINBERG** and **T. SCHUBB.**

[*Pittsburgh, Pa.*]

Starting out from the premise that paralysis agitans is due to hyperparathyroidemia, as advocated by several observers, we proceeded to prepare a parathyroidectin substance for the treatment of this condition. Experiments were conducted on rabbits and on goats. The two external parathyroid glands of the goat were removed, and after forty days the blood of the goat was withdrawn and glycerinized. The administration of this blood to Parkinsonian patients seems to show promising results. Further study of this method of therapy is now under way.

### 11 (1758)

## Typing of different strains of Bacillus botulinus by immunologic methods.

By **J. BRONFENBRENNER, M. J. SCHLESINGER** and **S. C. CALAZANS.**

[*From the Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston, Mass.*]

A number of strains of *Bacillus botulinus* isolated both abroad and in this country represent a fairly uniform group in so far as their cultural characteristics and the symptoms produced by their toxin are concerned. However, in respect to neutralization of toxin by antitoxin there exist two sharply distinct groups of this organism, thus suggesting that in fact we are dealing with two distinct antigens.

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[1] *loc. cit.*

In this attempt to find a method by which the existence of two antigenic varieties within the group of *Bacillus botulinus* could be established without the recurrence to the toxin-antitoxin test we found that neither the complement fixation nor the precipitation tests give satisfactory results. The agglutination test, however, offered a ready means for grouping as the results obtained with this test were in accord with those obtained by toxin-antitoxin tests. The agglutination test has permitted us to classify also such strains of *Bacillus botulinus* which have lost their toxicity under the conditions of test tube cultivation. As a control in all the above experiments we included a strain of *Bacillus sporogenes* and found that contrary to the statement in the literature all but one of the strains obtained by us from different laboratories in this country are free from *Bacillus sporogenes* contamination as judged by the above serologic tests.

### 12 (1759)

## The antiscorbutic potency of strawberries.

By **CLARENCE A. SMITH, OLAF BERGEIM,** and **PHILIP B. HAWK.**

[*From the Laboratory of Physiological Chemistry of Jefferson Medical College, Philadelphia, Pa.*]

Several guinea pigs were fed a diet of oats, milk, and hay until they were decidedly scorbutic. They were then given expressed strawberry juice, either fresh juice or juice previously boiled for five minutes. The symptoms of scurvy were overcome within seven days by the administration of ten c.c. per day of either boiled or unboiled juice. Strawberries, therefore, appear to be relatively rich in water-soluble C, and their content of this vitamine is not seriously decreased by five minutes boiling.

### 13 (1760)

## A modified anaphylactic reaction induced by X-rays.

By **R. G. HUSSEY** (by invitation).

[*From the Rockefeller Institute for Medical Research, New York City.*]

The following observations are of interest in connection with a theoretical consideration of the mechanism of classical serum anaphylaxis.

We have found it possible, as has v. Heinrich also, to modify the manifestations of anaphylaxis in guinea pigs by exposing them to X-rays. Guinea pigs weighing about 250 grams were given an intraperitoneal inoculation of 0.1 c.c. of horse serum (1 c.c. of a 1 – 10 dilution) for sensitization. Half of the number of sensitized animals were radiated immediately and then each day thereafter for 10 days. The X-rays were delivered from a Coolidge tube governed by a spark gap of 3 inches with 10 milliamperes of current. The distance from the anode to the surface of the animal's body was 6 inches and the total time of exposure was 10 minutes.

14 days after sensitization 0.1 c.c. or 0.01 c.c. of horse serum was inoculated into the jugular vein as an intoxicating dose. In the animals sensitized but not X-rayed, typical anaphylactic manifestations and usually acute death followed the inoculation with either amount of antigen. The X-rayed animals, on the other hand, showed either very slight or no objective anaphylactic manifestations. If, however, 4 weeks were allowed to elapse from the time of sensitization, then a similar amount of antigen inoculated intravenously, there was no difference in the behavior of X-rayed animals as compared with the controls. Further, it was found that radiation at any time other than during the incubation period did not induce a modified reaction.

With these facts established, we directed our attention to a study of the anaphylactic state of isolated tissue. It may be said that many investigators describe the anaphylactic reaction of isolated smooth muscle as an index of the reaction of the animal body as a whole. Indeed, this phenomenon is regarded by them as the most important evidence which indicates that the locus of antigen-antibody union is intracellular.

Female guinea pigs of about 225 grams were sensitized by an intrapleural inoculation of 0.1 c.c. of horse serum and subsequently treated as described in the original experiment. At intervals of 14 and 30 days following sensitization, the horns of the uteri were removed and segments of these treated in accordance with the principles of the well-known Dale method for studying the physiological behavior of isolated tissues. For each tissue preparation, we employed a suspension bath of 250 c.c. of oxygenated Locke's solution kept at a constant temperature of 38° C. When the muscle developed a satisfactory tone and rhythm, 0.2 c.c. or 0.5 c.c. of horse serum was added to the bath at a point which permitted uniform diffusion throughout the fluid before coming in contact with the tissue. The uteri of the sensitized and X-rayed animals reacted typically with maximal response just as did the uteri of the sensitized animals not X-rayed. The tracings of the uteri removed at both intervals show no essential differences.

The information furnished by the data presented we believe to have a direct bearing on the controversial point regarding the locus of antigen-antibody union which results in anaphylactic shock. The results of our experiments indicate that the anaphylactic reaction of isolated smooth muscle is not an index of the reaction of the animal as a whole. Also it is indicated that other factors than the reaction of sensitized smooth muscle should be taken into account in the statement of a theory concerning the mechanism of anaphylactic shock.

We have now in process an investigation in which we are determining the relation between the existence of free antigen and the presence of precipitins in the circulation of animals X-rayed and not X-rayed. The results of our experiments to date indicate that free antigen remains in the serum of X-rayed animals for a much longer period than is found in animals not X-rayed. A full report of these results, together with studies on passive anaphylaxis, will be published later.

### 14 (1761)

## Contribution to study of diphtheria toxin.

By **P. J. MOLONEY** and **L. HANNA** (by invitation).

[*From the Research Division, Connaught Antitoxin Laboratories, University of Toronto, Toronto, Canada.*]

The results reported are those of experiments planned to throw further light on the mechanism of toxin production by *B. diphtheriæ.*

An extended series of test-tube experiments was carried ou in which the Park 8 strain of the diphtheria bacillus was grow in broth and daily counts made of the number of viable organis (as estimated by the poured plate method), and at the same time the toxicity of the broth, free from organisms, was estimated.

The growth curve had the characteristics of those reported at various times in the literature for other organisms; *i.e.*, a period of logarithmic growth, and then a continued rise which in general reached a maximum in 48 hours, a sudden falling off of the number of viable organisms and then a more gradual decrease, with sometimes small increases in the number of viable organisms during the period of decrease similar to the observations of Graham-Smith[1] for staphylococcus. It is suspected that these small secondary increases may only be apparent, and are due to the method of counting. The toxicity of the broth was very low at the end of 24 hours (approx. 1 M.L.D. per 0.5 c.c.), with a small increase at the end of 48 hours and then a rapid increase (if the increase and decrease of the cells was characteristic), and after a maximum was reached a falling off in toxicity which was sometimes very sudden. This sudden falling off in toxicity has also been reported by Bunker.[2]

The hydrogen-ion concentration of the broth during growth showed an initial increase and then a gradual falling off; this agrees with the findings of Bunker[2] and Davis.[3] Bunker in his paper gives the limits of maximum toxicity at $P_H$ 7.8 − 8.25; contrary to this, good toxin has continued to be formed at $P_H$ 8.7

There are several ways in which the above results may be interpreted:

1. Reproduction and toxin production do not go on at the same time; a cell produces toxin when it is incapable of division; apparently it is not accumulation of toxin which inhibits reproduction since toxin—200 M.L.D's per c.c.—will support growth when replanted with *B. diphtheriæ*.

2. A non-toxic substance may be produced during the period of cell division which is transformed into toxin; or this non-toxic substance acts on some constituent of the broth and produces toxin. Non-toxic germ-free broths (after organisms had grown for less than 24 hours) which were sterilized in various ways—

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[1] Graham-Smith, *Journal of Hygiene*, 1920 (19).

[2] Bunker, *Journal of Bacteriology*, 1919, iv.

[3] Davis, *Journal of Bacteriology*. 1920, v.

heating at 56° C., berkefelding, and by adding various disinfectants, phenol, gentian violet, etc.—and then allowed to incubate at 37° C. for 5–6 days, failed to show any toxin production. Some experiments by Walbum[1] are interesting in this connection. Walbum deduced the existence of what he called pro-toxin; he injected a mixture of toxin plus peptone into a guinea pig in such amount that the pig died in five days; the same amount of peptone and toxin given separately to two other guinea pigs failed to kill. The experiments hardly seem conclusive. The peptone might conceivably injure the animal so that the toxin would kill more easily. He tried no experiments with other reagents than peptone, to check this point; and no experiments were undertaken to test the specificity of any toxin which may have been formed by this mixture.

3. There may be some sort of autolytic disintegration of the cells—so far there has been no evidence of this sort of mechanism. Organisms at different stages of life activity—after growing 1–2–4–6 days—were incubated at 37° C. for 6 days with saline and with distilled water. The clear sterile liquid in these several cases showed no toxin whatever. A possible objection to these experiments is that the digestion was not carried on in a colloidal substrate.

Appropos of this were the attempts made to prevent growth by planting a very large number of organisms, but these were unsuccessful, for no matter how large a number of organisms was planted there was always some growth. However, in every case so far tried the broth planted with a very large number of organisms showed less toxin than the same broth planted with a loopful. In some cases the difference was very marked. This difference in toxicity was apparently not due to absorption of the toxin by the organism; toxin allowed to stand in the ice chest with large numbers of organisms at different stages of life activity did not show any change in toxicity.

4. During the period of cell division a substance may be produced which acting on the non-viable cells produces toxin. The following results bear on this. After growing the organisms for less than 24 hours the whole was sterilized, in some cases by heating to

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[1] Walbum, *Zeit. Immunitatsforschung*, 1909 (3), originale.

56° C. and in others by adding disinfectants, and in each case allowed to incubate at 37° C. for 6 days. Sterilized thus by heat, by phenol and by gentian violet there is no evidence of toxin production. Experiments to test more thoroughly these various hypotheses are being planned. Details will be published in full elsewhere.

### 15 (1762)

## The change in reaction of dying tissue.

By WITHROW MORSE and H. C. VAN DER HEYDE.

[*From the Department of Physiological Chemistry,
School of Medicine, Morgantown, W. Va.*]

In the studies of tissue enzyme action which the senior writer has been making since 1910, there has always been the question of the stages immediately following the death of the tissue and also of the conditions of reaction of medium, which have been shown to regulate the character of the process, that is, the rate and equilibrium. An attempt was made by Strauss and Morse[1] to determine the reaction of medium in the kidney during hematogenous infarction brought on by ligation of the blood vessels and at the same time to determine whether autolysis proceeded or not. The former collaborator (D. C. S.) being called for service rendered it impossible to complete this series of studies. Earlier still[2] the Sørensen colorimetric method was employed in similar work, but the obvious difficulty of the time element involved in the dialysis inhibited very critical conclusions. Recently, Dernby[3] applied the Sørensen solutions with the Clark-Lubs indicators to the study of the problem, but the critical point regarding the inception of autolysis and the state of reaction of medium in the earliest stages was not investigated. In his third paper in the "Studies of Autolysis" [4] Bradley and collaborator found "soon after death" a reaction of $P_H = 7.00$ in normal liver, but inasmuch as beef and pig livers were used, it is probable that the source of supply was slaughter-house material as in previous work in the series, while there is nothing to indicate that the experiment with horse liver involved the incipient stages, so that no data seem to have been given which would permit one to judge how soon *post mortem* the experiments were conducted. Here, as in the studies of Dernby, the colorimetric method involving dialysis was employed (p. 263, l.c.). The writers are unable to find in biochemical literature any other studies of this nature and the following results of their work are presented with the view of interesting investigators in the problem where facilities are available for furthur work.

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[1] Strauss, D. C., and Morse, M. W., 1917, PROC. SOC. EXP. BIOL. AND MED. 1917, xiv, 171.

[2] Morse, M., *J. Biol. Chem.*, 1916, xxiv, 163.

[3] Dernby, K. G., *J. Biol. Chem.*, 1918, xxxv, 179.

[4] Bradley, H. C., and Taylor, J., *J. Biol. Chem.*, 1916, xxv, 261.

*Method.*—Guinea pigs were used, the pig being struck on the head with an iron mallet, laparotomy rapidly performed, the liver exposed and frozen *in situ* by means of an ethyl chloride spray. Then the liver was excised while the heart still beat, transferred to a cold mortar in an ice-bath at − 5° C., wherein it was ground to a snow. The temperature of the liver mass, however, varied but little from zero Centigrade; in this connection it is well to recall the findings of Foster and Moyle[1] in studies on muscle, where exposure to temperatures of from − 5° C. to − 8° C. led to relatively great development of acidity (lactic), the low temperature acting similarly to mechanical injury. The snow obtained in this way was transferred to the electrode vessel of the gas chain apparatus[2] and the temperature of the mass within the vessel was brought rapidly to about 20° C. by means of the warm hand. Potentiometer readings were made at frequent intervals and the readings followed for thirty-six hours. The contents of the vessel were agitated, moderately, by means of a stirrer, operated by a small motor. In order to check the apparatus, controls were run on Sørensen $NaOH - KH_2PO_4$ buffer mixtures, the variation from the expected being but slight in any one case. By this means, likewise, the time for reaching equilibrium was established as far as the phosphate-alkali mixtures were concerned, twelve minutes

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[1] Foster, D. L., and Moyle, D. M., *Biochem. J.*, 1921, **xv**, 334.

[2] The writers were permitted to use the apparatus belonging to the Department of Soils, West Virginia University.

PROTOCOL.

| Time. | Temperature. | Millivolts. | Calculated $P_H$ |
|---|---|---|---|
| I. 3:45 (Pig killed) | | (Saturated electrode) | |
| 3:51 (Transferred to electrode vessel) | | | |
| 3:54 | 13° C. | 420 | |
| 3:59 | 20 | 510 | 4.50 |
| 4:02 | 20 | 530 | 4.84 |
| 4:05 | 20 | 535 | 4.96 |
| 4:07 | 20 | 540 | 5.01 |
| 4:08 | 21 | 542 | 5.05 |
| 4:11 | 21 | 550 | 5.18 |
| 4:19 | 20 | 615 | 6.29 |
| 4:22 | 20 | 635 | 6.63 |
| 4:23 | 20 | 645 | 6.81 |
| 4:28 | 20 | 660 | 7.08 |
| 4:37 | 20 | 664 | 7.15 |
| 4:45 | 21 | 665 | 7.15 |
| 5:01 | 21 | 660 | 7.06 |
| 5:15 | 21 | 657 | 6.99 |
| 5:28 | 21 | 652 | 6.93 |
| 5:37 | 21 | 648 | 6.86 |
| 5:45 | 21 | 646 | 6.82 |
| 7:39 (Toluene added) | 21 | 630 | 6.55 |
| 9:05 (A.M.) | 19 | 640 | 6.40 |
| 11:15 | 22 | 590 | 5.90 |
| 1:45 P.M. | 21 | 587 | |
| 10:10 A.M. | 21 | 537 | 4.95 |
| 9:45 A.M. | 22 | 485 | |
| Discontinued. | | | |
| II. 10:14 Killed | | | |
| 10:20 Transferred to electrode vessel; heart in cadaver still beating. | | | |
| 10:23 | 20 | 370 | 2.09 |
| 10:25 | 26<br>(Vessel warmed) | 495 | 4.21 |
| 10:31 | 20<br>(Vessel cooled) | 490 | 4.15 |
| 10:35 | 21 | 497 | 4.27 |
| 10:39 | 21 | 500 | 4.32 |
| 10:44 | 21 | 500 | 4.32 |
| 10:55 | 22 | 502 | 4.35 |
| 11:11 | 22 | 497 | 4.26 |
| 11:29 | 22 | 496 | 4.25 |
| 11:40 | 22 | 495 | 4.23 |
| 1:15 | 23 | 622 | 6.39 |
| 1:40 | 22 | 641 | 6.73 |
| 1:47 | 22 | 643 | 6.76 |
| 3:38 | 23 | 626 | 6.46 |
| 4:05 | 23 | 620 | 6.80 |
| 4:45 | 22 | 605 | 6.11 |
| 5:15 | 23 | 590 | 6.20 |
| 8:45 A.M. | 20 | 520 | 4.67 |
| 8:50 | 20 | 527 | 4.79 |
| 9:05 | 20 | 528 | 4.81 |
| Discontinued. | | | |

being necessary. This figure is taken as a basis for the tissue work. The writers are unable to determine any factor in the tissue which may prolong the period of reaching equilibrium and while it is possible to explain the results obtained, as having to do with inequilibrium, the burden of proof is rather upon this aspect of the question, for one must show why liver tissue should demand more time for reaching equilibrium than the buffers.

The protocols following are those of two experiments. A third was conducted with practically identical results:

The results are striking, the reaction of the tissue being decidedly acid at the first reading, taken within five minutes after the time of excision of the liver. Then there is a slow fall to neutrality, which is reached within about 45 minutes. A rise ensues, which continues for a considerable length of time, over 24 hours at least.

The meaning of these findings is not clear, but they may be due to the fact that acid is produced at first in an explosive way, a conclusion which is justified by the studies of Fletcher,[1] who found that one fifth of the $CO_2$ produced by an excised muscle arose in the earliest stages; by the studies of Fletcher and Hopkins,[2] who found, always, in dying tissues lactic acid; and by the investigations of Foster and Moyle,[3] who found 0.218 per cent. lactic acid developed in injured muscle (minced) as compared to uninjured muscle 12 days at 0° C., 0.017 per cent. Secondly, the buffer action of the proteins, etc., in the tissue may exert its effect, causing a "fixing" of the free acid, but finally this effect is nullified by a saturation of the buffers and a rise in free acid begins.

If these results are free from criticism, a more substantial basis for the conception of how autolysis proceeds is available. Bradley showed in his first series of studies that the proteins of the substrate in autolysis became altered in some way whereby they became more digestible in tissue hydrolysis under the influence of the tissue protease and Dernby virtually substantiates these findings. The older work of Dochez, of Hedin and of Rowland point to this conclusion and the interpretation of relation of reaction to substrate is in keeping with the recent studies of Northrup,

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[1] Fletcher, W. M., *J. Physiol.*, 1902, xxviii, 354.

[2] Fletcher, W. M., and Hopkins, F. G., *J. Physiol.*, 1907, xxxv, 247.

[3] *loc. cit.*

Falk and others upon different material. If we assume that the alkalinity of the tissue is slowly changed to acid reaction, it is difficult to see how low hydrogen ion concentration can operate to render the tissues more digestible, whereas a high degree of acid, such as we have found developed in the liver in the present study, may well be imagined to exert a profound influence upon the character of the proteins of the liver, for this concentration resembles that of gastric juice, especially that of the young subject,[1] where proteins are digested rapidly.

Since the above statements were written, the electrometric method has been checked by the Sørensen colorimetric method supplemented by the indicators of Clark and Lubs. Practically identical results have been obtained with both liver and kidney. The details of the method, with results and discussion, will be given in another place under the following title: "Further Studies on the Reaction of Dying Tissues," by Withrow Morse and R. Goldberg. The question will be raised therein, whether the suggestion made by Paul Erlich ("Die Aenaemie") that the reaction of the nucleus is acid, is applicable here.

### 16 (1763)

## The cure of infantile rickets by sunlight as demonstrated by a chemical alteration of the blood.

By **ALFRED F. HESS** and **P. GUTMAN.**

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

It has been shown by one of us (A. F. H.) that the rickets of infants can be cured merely by frequent exposures to the sun's rays.[2] Animal experiments carried out in this laboratory confirmed these clinical observations. They clearly demonstrated that rickets could be either prevented or brought about in rats fed a standard diet, according to whether they were subjected for short periods to the sun's rays or were kept at all times in the dark. The present report adds substantiation from the chemical side to the clinical and anatomical evidence brought forward in the previous papers.

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[1] McClendon, J. F., *Amer. J. Physiol.*, 1915, xxxviii, 191.

[2] Hess, A. F., and Unger, L. J., PROC. SOC. EXPER. BIOL. AND MED., 1921, xviii, 298.

Hess, A. F., and Unger, L. J., *J. A. M. A.*, 1921, lxxvii, 39.

In a recent article Howland and Kramer[1] have shown that the inorganic phosphorus of the serum of infants suffering from active rickets is reduced, and that during the process of healing, especially upon the administration of cod liver oil, the phosphorus content gradually rises to normal. In view of the marked clinical improvement following sun treatment, it seemed of interest to ascertain whether this procedure was accompanied by a chemical alteration of the blood. For this purpose the rapid colorimetric method of Bell and Doisy[2] was used, in which the color is developed in protein-free filtrates through the reduction of phosphomolybdic acid by hydroquinone in alkaline sulphite solution. Special attention was paid to the inorganic phosphorus of the blood, although in many instances the so-called acid-soluble and total phosphorus was also estimated. It will be seen from the accompanying chart that the normal figures for inorganic phosphorus are sufficiently constant to render this test of clinical value (Table I).

TABLE I.

BLOOD PHOSPHORUS IN NORMAL INFANTS.

| Name. | Age, Mos. | Inorganic. | Acid Soluble. | Total. | Name. | Age, Mos. | Inorganic. | Acid Soluble. | Total. |
|---|---|---|---|---|---|---|---|---|---|
| | | Mg. P. per 100 c.c. Blood. | | | | | Mg. P. per 100 c.c. Blood. | | |
| M.K. | 6 | 4.40 | 14.5 | | P.D. | 10 | 4.48 | 18.5 | 45.8 |
| B.S. | 3 | 4.33 | 16.2 | 38.3 | S.D. | 10 | 4.31 | 17.2 | 46.3 |
| F.M. | 15 | 4.60 | 17.8 | 41.6 | B.H. | 12 | 4.60 | 18.1 | 53.5 |
| B.S. | 3 | 4.42 | 17.2 | 56.8 | A.M. | 11 | 4.76 | 13.0 | 37.8 |
| H.H. | 11 | 4.80 | 25.9 | 43.0 | H.M. | 7 | 4.10 | 16.0 | 68.2 |
| G.H. | 8 | 4.69 | 16.4 | 34.3 | B.F. | 6 | 4.00 | 18.9 | |
| M.D. | 4 | 4.65 | 17.2 | 37.5 | A.R. | 13 | 4.44 | 18.7 | 52.5 |
| J.F. | 18 | 4.44 | 16.0 | 49.6 | D.B. | 9 | 4.17 | 18.5 | 39.4 |
| B.R. | 11 | 4.39 | 16.8 | 43.6 | H.R. | 13 | 4.61 | 19.6 | 40.5 |
| W.L. | 8 | 4.10 | 14.9 | 67.1 | S. F. | | 4.00 | | |
| J.R. | 6 | 4.10 | 15.3 | 63.5 | H. B. | | 4.40 | | |
| B.B. | 6 | 4.05 | 17.5 | — | L. S. | | 4.00 | | |
| A.S. | 6 | 4.25 | 15.0 | 54.7 | S . B. | | 4.80 | | |
| A.A. | 2 | 4.17 | 15.9 | 35.0 | M. G. | | 4.34 | | |
| M.C. | 11 | 4.14 | 18.7 | — | | | | | |
| T.S. | 10 | 4.20 | 18.3 | 41.4 | | | | | |

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[1] Howland, J., and Kramer, B., *Am. J. Dis. Child.*, 1921, xxii, 105.

[2] Bell, A. F., and Doisy, E. A., *J. Biol. Chem.*, 1920, xliv, 55.

The infants were placed in the direct sunlight for a half hour to several hours, the period varying according to the intensity of the sun and the sensitiveness of the skin. Previous to treatment the majority of infants showed the usual clinical symptoms of mild rickets and the characteristic signs on x-ray examination. Such, however, was not invariably the case; it has been our experience that infants may manifest the classical signs of rickets, accompanied by a low inorganic phosphate content of the blood, and, nevertheless, show apparently normal epiphyses at the wrists and other joints. In the course of the sun treatment the babies became markedly tanned, the rachitic signs diminished or disappeared, and the general condition improved.

The accompanying table, which shows successive examinations of the blood, requires little interpretation (Table II). It will be seen that the inorganic phosphorus of the rachitic infants steadily increased from month to month, starting generally below 3.5 mg. per 100 c.c. of blood and gradually being restored to the normal level which must be considered about 4.0 mg. On the other hand, the results of the determinations of "acid-soluble" and of total phosphorus are not sufficiently definite to warrant deductions. There were no alterations in the diet throughout these periods, the infants receiving the usual milk formulas; all were given orange juice daily, the older children getting cereal in addition.

TABLE II.

BLOOD PHOSPHORUS OF RACHITIC INFANTS TREATED WITH SUNLIGHT.

| Name. | Age (mos.). | Inorganic P. | | | | | Acid Sol.[1] | Total.[1] |
|---|---|---|---|---|---|---|---|---|
| | | 6/22 | 7/21 | 8/11 | 9/16 | 10/18 | | |
| F.R...... | 7 | 2.80 | 3.75 | 4.14 | 4.13 | | 15.7 | 43.6 |
| P.F...... | 5 | 3.7 | 3.4 | 4.16 | 4.22 | | 23.0 | 41.0 |
| I.A....... | 13 | 2.77 | 2.75 | 3.53 | 4. | | 15.2 | 43.6 |
| M.L...... | 8 | 3.1 | 3.18 | 3.75 | 4.28 | | 15.0 | 36.0 |
| R.M...... | 15 | 3.0 | 3.02 | 3.16 | 3.87 | | 14.5 | 44.6 |
| C.M..... | 37 | 3.4 | | | 3.77 | 4.3 | 19.3 | 52.0 |
| T.M..... | 7 | 3.0 | | | 3.9 | 4.0 | 16.6 | 38.5 |
| M.E..... | 18 | | 4.0 | | | 3.77[2] | 15.2 | 56.8 |
| H.G..... | 15 | 4.6 | | | | 3.69[2] | 15.9 | 39.0 |

It is evident that sunlight not only brings about a cure of the rachitic lesions, but in so doing occasions chemical changes in the blood similar to those noted when the cure is effected by cod liver oil. This is of interest as affording testimony that the curative process occasioned by these divergent therapeutic agents is fundamentally the same. These observations establish a chemical basis for heliotherapy in rickets. They furnish also, as far as we know, the first definite evidence of metabolic change in the animal body brought about by the solar rays.

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[1] Tests made with blood used for the first inorganic P. determination.

[2] Previous to treatment.

### 17 (1764)

## Dissociation of microbic species.

## II. Mutation in pure-line strains of the bacillus of rabbit septicemia.

By PAUL H. DE KRUIF.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

The coexistence of two distinctly different types of microbe in cultures of the rabbit septicemia bacillus has been reported in a previous paper.[1] These varieties, once separated, appear to breed true to type for many passages. The organisms have been designated as types D and G. Type D is very virulent for rabbits, grows diffusely in liquid media, and yields highly fluorescent, rather opaque colonies on serum agar. Type G is of extremely low virulence, exhibits a granular sedimenting growth in fluid media, and grows in the form of translucent, non-fluorescing colonies on serum agar. The two types show no noticeable differences in morphology or in fermentation reactions. Immunization and agglutination reactions indicate their antigenic community.

It seemed necessary to determine whether the two varieties coexist in cultures isolated from infected rabbits or whether one variety arises from the other. Type D (virulent) is the microbe invariably obtained from the naturally infected rabbit. Type G has only been found after artificial cultivation has been carried on for some time. But since the primary isolations were made from colonies which conceivably might arise from two or more organisms, it would be unjustifiable to conclude that the original type D had changed into the microbe of the G variety. Consequently, 8 pure-line strains were isolated from a D culture by the Barber method. Single cells, removed from three-hour cultures, were planted in undiluted rabbit serum. The percentage of positive cultures obtained by performing the entire operation in serum was much higher than when broth was employed.

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[1] *Jour. Exper. Med.*, 1921, xxxiii, 773.

The resulting pure-line strains were planted daily in undiluted rabbit serum. Tests were carried out to determine the conditions under which the low-virulent type G makes its appearance. The method of detection of this type consisted in streaking the test material upon the surface of 10 per cent. rabbit serum agar. The G type colonies can be readily distinguished from those of the strongly fluorescent type D. With proper attention to the technique of streaking, quantitative estimates of the proportionality between the D and G varieties can be made.

In undiluted rabbit serum, with daily transplant, type D breeds true for long periods of time. The G variety has seldom been observed to arise under these conditions. In plain broth, transplanted daily, a few G colonies have been detected after 25 passages. On the other hand, when 3 or 4 days are allowed to elapse between transplants in this medium, many colonies of this type make their appearance on the serum agar sub-plates.

This observation led to the following experiment. 0.05 c.c. of a pure-line strain, type D, was seeded into tubes of plain broth and of undiluted rabbit serum. The tubes were placed at 37° C. and a loopful of the material from each tube was streaked at 12-hour intervals on rabbit serum agar plates. In the sub-plates from undiluted rabbit serum no G colonies were detected during incubation for 109 hours at 37° C. In the plain broth, G colonies began to appear at 48 hours, and had reached a concentration of 50 per cent. of the total organisms in 109 hours. These G colonies, fished from serum agar plates, remained true to type for over 50 passages, showing no tendency to revert to the parent D form, even when returned to undiluted rabbit serum. All of the pure-line strains under study have been found to undergo this mutation when allowed to stand in plain broth, but do so with varying degrees of rapidity and completeness.

It was considered probable that filtrates from D cultures might hasten the D ⇀ G transformation. Accordingly, cultures of 6, 24, 48, and 72 hours were filtered through Berkefeld candles. After sterility had been proved, 0.05 c.c. of pure-line strain B–$D_2$ was seeded into 10 c.c. of each of the above filtrates, into controls of sterile broth, and into undiluted rabbit serum. The tubes were incubated at 37° C. and streaked on serum agar plates at intervals up to 176 hours. Contrary to expectation, the number of G colonies arising in the 6- and 24-hour filtrates was extremely small, and comparatively few appeared in that of 48 hours. In the 72-hour filtrate G colonies appeared at a rate and in a concentration approximately parallel to that of the control broth. The mutation had reached 50 per cent. in 176 hours. In the undiluted rabbit serum no G colonies appeared at any time during the experiment. It would seem, then, that early filtrates from D cultures are antagonistic to the D ⇀ G mutation.

The $C_{H+}$ of the broth seems, within limits, to have no effect upon the rapidity of the mutation. If anything, an acidity $> P_H = 7.0$ retards the process. Tests were made down to $P_H = 6.0$, beyond which point it is difficult to obtain growth.

An effort was made to discover the constituents of plain broth that encourage the tendency of type D to change to the G variety. Pure-line strains of the former were planted in beef infusion, and in various concentrations of peptone (Fairchild). The $P_H$ of all the media was adjusted to 7.4. It was found that little or no mutation occurred in the beef infusion up to 200 hours at 37° C. In 0.5 to 1.0 per cent. concentrations of peptone some D ⇀ G change was noted. But when higher concentrations, up to 20 per cent., were employed, a very rapid mutation set in, reaching 90 per cent. of the total organisms in 96 hours. This was true even when the peptone solutions were made up to volume with beef infusion. Control tubes of undiluted rabbit serum and of beef infusion showed one or two G colonies at 120 hours, but none after 144 hours or after 8 days. This experiment indicates that peptone in suitable concentrations accelerates the D ⇀ G process.

The G colonies arising in these experiments, and sub-cultured to undiluted rabbit serum, were frequently tested for their distinguishing characters, *i.e.*, low virulence and granular growth in fluid media. They were found in every case to satisfy these criteria. What is more, the acid agglutination point is distinctly different to that of the D variety. It is in the nature of a physical constant for each type, and is an important differential criterion. All of these characters persist throughout many passages in undiluted serum, a medium markedly antagonistic to the original change. It cannot be said that the presence of the peptone causes the mutation D → G, since the change occasionally occurs, though very rarely and in small amount, in undiluted rabbit serum. On the other hand, the presence of peptone in suitable concentration greatly accelerates a reaction toward which a tendency already exists. It is of interest to note that four pure-line strains, kept on ice in undiluted rabbit serum for three months without passage, showed no evidence of the appearance of G colonies.

### 18 (1765)

## Dissociation of microbic species.

## III. Differentiation of microbes D and G by acid agglutination.

By PAUL H. DE KRUIF.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

Granular sedimenting growth in liquid medium is one of the principal characters differentiating microbe G (bacillus of rabbit septicemia) from its parent D form. Type G exhibits the granular appearance not only in plain broth, but in serum broth and in undiluted serum as well. This fact led to the examination of the comparative acid flocculation points of the two types. The method used was that of Michaelis, later described in full by Beniasch.

The suspensions of types G and D were prepared by washing the sediments from 5 per cent. serum broth cultures in large volumes of distilled water. After this procedure had been repeated four times, the final suspensions were carefully brought to equal turbidity. Prepared in this way, the G type suspension shows a stability equal to that of D.

The tests for acid agglutinability were carried out with mixtures of Na lactate-lactic acid, range $P_H = 4.7$ to $P_H = 2.4$, and with Na acetate-acetic acid, range $P_H = 5.6$ to $P_H = 3.2$. The mixtures of these buffer series with the microbic suspensions were incubated at 43° C. for 16 hours. Readings were taken at the end of this time. A distinct difference in acid agglutination optimum for the two types was observed. The optimum for type G in general occurs at a range between $P_H = 4.7$ and $P_H = 4.0$. Type D, on the other hand, shows complete sedimentation between $P_H = 3.5$ and $P_H = 3.0$. Many strains of the two types have been examined with invariably the same result. This observation furnishes an important differential criterion for the two varieties. The constancy of the acid agglutination optimum for type D is very strict. That for type G is slightly less so, but the variation is never so great as to cause it to be confused with D.

### 19 (1766)

## Dissociation of microbic species.

## IV. Factors influencing the acid agglutination optimum of types D and G.

By **PAUL H. DE KRUIF.**

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

It is generally supposed that the acid flocculation optimum of bacteria is referable only to the $C_{H+}$ and is not influenced by the character of the buffer salts or the anion of the acid. This interpretation is questionable in the light of the following facts. Microbes D and G were tested against a glycocol-HCl buffer series, range $P_H = 3.0$ to $P_H = 1.2$. The same suspensions were tested simultaneously with the Na lactate-lactic acid and the Na acetate-acetic acid series employed in the experiments described in the preceding paper. The results are presented in the following table.

This experiment indicates that other factors besides the $C_{H+}$ are important in the interpretation of the acid agglutination point of the organisms in question. For example, complete flocculation of type G occurs at $P_H = 3.0$ in the glycocol HCl series, while no flocculation whatever was observed at the same $P_H$ in the Na lactate-lactic acid series. It would appear that either the $Cl^-$ of the acid, or the glycocol possessed the property of broadening the optimum zone or of shifting it toward a higher $C_{H+}$.

ACID AGGLUTINATION OF TYPES G AND D IN VARIOUS BUFFER SERIES.

| Buffer Series. | $P_H$ Range. | Complete Flocculation. | |
|---|---|---|---|
| | | G. | D. |
| Glycocol HCl | 3.0 to 1.2 | 3.0, ++ at 2.8 | 3.0 to 2.4 |
| Na lactate-lactic acid | 4.7 to 2.4 | 4.7 to 4.1 | 3.5 to 3.3[1] |
| Na acetate-acetic acid | 5.6 to 3.2 | 4.7 to 3.8 | 3.5 to 3.2 |

This and other considerations led to experiments which suggest an explanation for the granular growth of microbe G in plain broth. Washed suspensions of this organism are strongly agglutinated by beef infusion between $P_H = 7.4$ and 6.8. This range represents the $C_{H+}$ occurring during the growth of type G in broth. On the other hand, peptone (Fairchild) and $Na_2HPO_4$, the other constituents of broth, agglutinate type G very little or not at all in this acidity.

Types G and D were next subjected to tests with varying amounts of beef infusion, which were adjusted to varying acidities, from $P_H = 7.5$ to $P_H = 2.0$. The dilutions of beef infusion were varied from 1–2 to 1–40. Each dilution was tested over the range of acidity just mentioned. Incubation at 43° C. for 16 hours. It was found for type G that as the acidity increases, down to $P_H = 4.5$, the amount of beef infusion necessary to cause complete agglutination becomes less. At $P_H = 4.5$ to $P_H = 4.0$, complete flocculation occurs with traces of beef infusion or with none at all. But as the acidity is increased beyond this point, that is, at $P_H = 4.0$, *increasing* amounts of the beef infusion are necessary to produce this result. The same phenomenon is observed for type D, the only difference being that the complete flocculation of this variety by a given concentration of beef infusion demands a higher $C_{H+}$ than in the case of type G. For type D, the optimum zone lies between $P_H = 4.0$ and $P_H = 3.0$. Beyond this, in the direction of greater acidity, more and more beef infusion is necessary to produce complete flocculation.

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[1] No flocculation at 2.7 and 2.4.

It will be observed that the range of $C_{H+}$ at which the smallest amount of beef infusion is required is for each type precisely the zone of the acid agglutination recorded in the preceding paper. This experiment indicates that the beef infusion, *per se*, does not cause the agglutination. It merely widens the acid agglutination zone. This would seem to throw light upon the mechanism of the granular growth character of type G in plain broth.

Suspensions of types D and G were similarly tested against decreasing concentrations of peptone at varying $C_{H+}$. In these experiments the results were of a different nature, as might have been expected from the failure of peptone to agglutinate type G at $P_H = 7.5$ to $P_H = 6.8$. In the case of peptone, the optimum for type G lies at a range between $P_H = 3.0$ and $P_H = 2.5$. That for D, at $P_H = 2.5$. Peptone, therefore, seems to shift the optimum zone in the direction of a higher $C_{H+}$, an effect analogous to that observed in the glycocol-HCl buffer mixtures. In the case of microbe D, strong concentrations of peptone (1–2 and 1–4) actually suppress flocculation completely at $P_H = 3.0$. This effect is analogous to the pre-zone phenomenon in immune reactions, since for the higher dilutions of peptone at this $C_{H+}$, complete agglutination readily occurs.

It would appear from the foregoing that while the flocculation in all cases under consideration is due to H-ions, at the same time other factors, such as glycocol, peptone or beef infusion, either shift or broaden the acid agglutination optimum.

### 20 (1767)

## Dissociation of microbic species.

### V. Further considerations in regard to the virulence of microbes D and G.

By **PAUL H. DE KRUIF.**

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

The wide variations in virulence between microbes D and G, bacillus of rabbit septicemia, has been demonstrated in the first paper of this series.[1] Microbe D, the type found in natural infections, is possessed of powerful invasive properties, while its mutant form G is characterized by very low virulence.

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[1] *Jour. Exper. Med.*, 1921, xxxiii, 773.

The fixity of the character of high virulence for type D is demonstrated by the following experiment. Strain R-19, type D, was tested a few days after its isolation from a rabbit dead of broncho-pneumonia. The test was carried out by injecting high dilutions of a six-hour serum broth culture intrapleurally into young rabbits of 600 grams weight. The strain proved itself fatal in $10^{-8}$ c.c. of the serum broth culture. This culture was transplanted every seven days on serum agar. Tests made one and three months after the first experiment indicated its virulence to be still of the same titer.

The individuals of a given strain of type D appear to differ very little in the characteristic of virulence. Six pure-line strains, isolated by the Barber method from stock strain R-15, were tested for virulence by the method just described. All were fatal in dose of $10^{-6}$ c.c.

The virulence of type D not only remains constant during passage on serum agar, but persists under conditions that may be considered as distinctly unfavorable. For example, a pure-line strain of type D was planted in plain broth. It was allowed to remain at 37° C. for 9 days and 12 hours without further transplantation. At the end of this time a culture was streaked on a serum agar plate. Marked D → G mutation had occurred, counts showing D = 40, G = 60. A colony of each type was fished into serum broth tubes. These were incubated for the usual time, diluted appropriately, and injected into two series of rabbits of 600 grams weight. The D culture, injected over a range from $10^{-7}$ to $10^{-1}$ c.c., proved fatal in every case. The G culture, on the other hand, failed to provoke a noticeable effect, even when 0.5 c.c. of whole culture was injected.

It has been remarked in the second paper of this series that pure-line strains of microbe D may mutate during daily passage in plain broth. A pure culture of type D, virulence $10^{-6}$, was transplanted daily in plain broth for 25 passages. Its virulence remained constant during this time. At the 30th passage a few G colonies were observed on the serum agar sub-plates. In two months, sub-cultures on serum showed a large preponderance of G colonies. The virulence had fallen to $10^{-4}$ c.c. Twenty-five days later, no D colonies could be demonstrated. The mutation $D \rightharpoonup G$ was complete, or type G had completely outgrown type D. 0.1 c.c. of culture failed to produce a fatal effect. The attenuation of this culture is to be referred to the gradual replacement of the primordial D by the mutant G form. It is possible to predict the virulence from the relative preponderance of the two types, as evidenced by colonies on serum agar plates. It is possible to procure sub-cultures of very high or of very low virulence by selection of one type or the other, so long as any of the D type remain.

While the virulence of microbe G is very low, 1.0 to 2.0 c.c. of whole culture may occasionally produce fatal infections, especially in young rabbits. The organisms recovered from such animals at necropsy retain their granular growth character, but may gain perceptibly in virulence. After three animal passages, a type G culture has been observed to reach a virulence of $10^{-4}$ c.c. But despite this increase in invasive power, the non-fluorescence of its colonies persisted, its granular growth character intensified, and its acid agglutination optimum rose to $> P_H = 5.6$. It is apparent from this experiment that it is unsafe to state that low virulence goes invariably hand in hand with the other characters gained in mutation. Experiments are under way to determine whether this artificially produced virulence of type G is permanent or evanescent.

The route of infection is important in determining the ability of the low-virulent type G to gain a foothold in the animal body. A culture of microbe G which produced no perceptible effect when injected into rabbits *intrapleurally* in dose of 1.0 c.c. gave rise to abscesses when injected *subcutaneously* in 0.1 c.c. These lesions remained sharply circumscribed, but the type G organism could be recovered from them for several weeks after the appearance of the abscess.

The phenomenon of vicariously greater susceptibility to subcutaneous injection would seem to be due to the rapidity with which phagocytes are mobilized against type G when this organism is injected into the pleural cavity. Within 6 hours after intrapleural injection of 1.0 c.c. of serum broth culture of type G, no free organisms could be demonstrated in the aspirated fluid. Polymorphonuclear cells were present in large numbers and phagocytosis was intense. The virulent type D, on the other hand, gains its foothold primarily by reason of the late appearance of phagocytes following its intrapleural injection.

The occurrence of the low-virulent type G would seem to afford an excellent opportunity for the investigation of the properties or products of secretion which give the parent D type its characteristic of high virulence.

21 (1768)

## II. The prevention of the development of rickets in rats by sunlight.

By **P. G. SHIPLEY, E. A. PARK, G. F. POWERS, E. V. McCOLLUM** and **NINA SIMMONDS.**

[*From the Department of Pediatrics, Johns Hopkins University, Baltimore, Md., Dept. of Pediatrics, Yale University, New Haven, Conn. and School of Hygiene, Johns Hopkins University, Baltimore, Md.*]

In June, 1919, Huldschinsky[1] reported that the ultraviolet ray exerted a curative action in rickets. The criterion on which he relied was the evidences furnished by the X-ray of calcium deposition at the ends of the long bones. He found that there were definite signs of calcium deposition after four weeks of treatment and that at the end of eight weeks healing was almost complete. In May, 1920, Huldschinsky[2] again reported the curative effects of treatment with the ultraviolet ray in rickets in a series of thirty children, aged between one and one half and six and one half years, who exhibited all clinical manifestations of the disease. In all, healing was accomplished after twenty-two to twenty-six treatments covering a period of two months. In April, 1920, Putzig[3] corroborated the findings of Huldschinsky. He obtained cures by means of the quartz lamp in premature infants suffering from rickets. In July, 1920, Riedel[1] further confirmed Huldschinsky's findings in a series of one hundred children suffering from rickets. In June, 1921, Hess[2] confirmed Huldschinsky's findings in a series of six cases.

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[1] Huldschinsky, K., *Deutsch. Wchnschr.*, 1919, xlv, 712.

[2] Huldschinsky, K., *Ztschr. f. orthop. Chir.*, 1920, lxxxix, 426.

[3] Putzig, H., *Therap. Holbmonatschr.*, 1920, viii, 234.

The favorable influence of sunlight in rickets has been recognized by some students of the disease for a long time, notably by Palm[3] (1890), and experimental evidence of its beneficial effect on mineral metabolism in the puppy has been furnished by Raczynsky[4] in 1912. Huldschinsky made use of sunlight together with the ultraviolet ray in two cases of his series and Riedel relied on treatment with sunlight in some of his cases, supplementing with the quartz lamp ray only on sunless days. Hess[5] was the first, so far as we are aware, to demonstrate, by means of the radiograph, that sunlight alone exerts the same curative action as the ultraviolet ray. All the investigations which have been made up to the present time in regard to the curative effects of both the ultraviolet ray and sunlight in rickets have been made on human subjects of the disease and all the evidence has been furnished by means of the radiograph. In order to satisfy ourselves concerning the action of light in rickets as well as actually to see the changes produced in the bones we performed the following experiments.

Eighteen rats about six weeks old and weighing between forty and fifty grams were placed on diet 3,143 which, as previous experience[6] has shown, produces rickets comparable in every respect to the rickets manifesting itself in human beings. The ration has the following composition:

| | |
|---|---|
| Wheat | 33.0% |
| Maize | 33.0 |
| Gelatin | 15.0 |
| Wheat gluten | 15.0 |
| NaCl | 1.0 |
| $CaCO_3$ | 3.0 |

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[1] Riedel, G., München. med. Wchnschr., 1920, lxvii, 838.

[2] Hess, A. E., and Unger, L. J., *Am. J. Dis. Child.*, 1921, xxii, 186.

[3] Palm, T. A., *The Practitioner*, 1890, xlv, 270–279 and 321–342.

[4] Raczynski, J., *Compt. rend. de L'Association Internationale De Pédiatrie*, Paris, 1913, p. 308.

[5] Hess, A. F., and Unger, L. J., *J. A. M. A.*, 1921, lxxvii, 39.

[6] McCollum, E. V., Simmonds, Nina, Shipley, P. G., and Park, E. A. *J. Biol. Chem.*, 1921, xlvii, 507.

It contains nearly twice the optimal content of calcium, and is decidedly below the optimum in its content of phosphorus and in fat soluble A. Otherwise, it is well constituted.

Twelve rats placed upon this diet were sent to New Haven, there to be exposed to sunlight. The remaining six rats were retained in Baltimore to be kept as control animals under ordinary laboratory conditions in a large, well-ventilated room completely screened with windows of ordinary glass. The animals treated with the sunlight were divided into two groups and placed in fairly large wire mesh cages. Each clear day the cages were carried out of doors and placed in the sunlight. At first, the weather being warm, the rats were exposed to the sunlight for two short periods of twenty minutes each. Soon, however, the periods were lengthened to six or even more hours. During the experimental period, which covered between sixty-two and sixty-seven days, the rats were exposed to the sunlight on every day except nine. The total exposure to sunlight during the experimental period varied between two hundred and forty-two and two hundred and seventy-three hours. The average daily exposure was four hours.

When first exposed to sunlight, the albinos developed conjunctivitis; the ears of all, in particular the albinos, began to peel; the skin of the tails became sunburned and rough; the hair of some of the albinos acquired a yellowish tint. Long before the experiments were completed it became evident that the animals treated with sunlight were not developing rickets. Though they did not grow normally, they remained extremely active, climbing and darting about the cages. Toward the end of the experiments the males became sexually active; one of the females became pregnant.

The control rats, killed at the expiration of two months, showed all the gross and microscopic evidences of rickets, the characteristic deformities of the thorax, enlargement and distortion of the costochondral junctions, fractures of the shafts, enlargements at the wrists, ankles and knees, and the ends of all the long bones. The bones cut with diminished resistance. On section a deep rachitic metaphysis entirely free from calcium was exposed. Into it the proliferative cartilage extended in irregular prolongations. The trabeculæ were surrounded with broad zones of osteoid.

The rats exposed to the sunlight, on the other hand, showed none of the evidences of rickets. The thorax was not deformed; the costochondral junctions were normal. There were no fractures of the ribs. The ends of the long bones were not enlarged. The long bones cut with great resistance. On microscopic examination the cartilage was normal. The proliferative zone was completely calcified. The trabeculæ were completely calcified. The condition found was normal except that both microscopically and grossly the bone was more delicate than in the rat of corresponding age reared on satisfactory diets. Though the sunshine completely prevented the development of rickets, it did not entirely compensate for the deficiency of phosphorus in the diet, either as regards the growth and development of the rat as a whole or of the skeleton.

There were some noteworthy findings outside the skeleton. An abundance of fat was present. In the control rats the fat was scant. The thymus was only partially involuted. In the control rats it was completely involuted. The spleen was not enlarged.

## DISCUSSION.

Sunlight effectually prevents the development of rickets in the rat. We have already shown,[1] as has also Pappenheimer, that cod-liver oil prevents the development of rickets in the rat. As nearly as we can judge from the radiographs furnished by Huldschinsky and others the mode of healing at the cartilage-shaft junction induced by the ultraviolet ray (sunlight) is exactly analogous to that which occurs after the administration of cod-liver oil, as determined by Howland and Park.[2] The time relations are also similar. Huldschinsky found that the ultraviolet ray produced definite evidences of healing at the end of four weeks, and at the end of two months almost complete healing. Howland and Park found that cod-liver oil first gave rise to evidences of healing at the junctions of the cartilage and shaft of the long bones three weeks after the administration was begun and that at the end of about two months the calcification of the diseased ends of the shafts seemed to be complete. Moreover, as the result of the gross and histological examinations made on the rats fed the rickets-producing diet 3,143 but exposed to sunlight it is possible to say that the changes produced by sunlight in the skeleton do not differ in any important respect from the changes produced when the animals are kept in room light but on a diet supplemented by cod-liver oil. Cod-liver oil contains something which is essential for optimal cellular function. Light also contains something which is essential for optimal cellular function. Cod-liver oil or light when made available to an organism previously deprived of either permits the organism to put into successful operation adaptations or defense mechanisms which otherwise would have been ineffectual. Neither cod-liver oil nor light meets the defects in the composition of the diet directly by supplying to the body either calcium or phosphorus but meets them indirectly by so raising the potential of cellular activity as to secure the most efficient utilization possible of those substances available in the body which are directly or indirectly concerned with ossification and calcification.

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[1] Shipley, P. G., Park, E. A., McCollum, E. V., and Simmonds, Nina. PROC. SOC. EXPER. BIOL. AND MED., xviii, 227, 1921.

[2] Howland, J., and Park, E. A., *Arch. Pediat.*, 1920, xvxvii, 411.

Howland, J., and Park, E. A., *Bull. Johns Hopkins Hosp.*, November, 1921. (To be published.)

## ABSTRACTS OF THE COMMUNICATIONS PACIFIC COAST BRANCH.

### Thirtieth meeting.

*Berkeley, California, October 15, 1921.*

22 (1769)

### The production of tyrosine by a putrefactive anaërobe.

By **IVAN C. HALL** and **FLORENCE FINNERUD.**

[*From the Department of Bacteriology and Experimental Pathology, University of California, Berkeley, California.*]

In caring for our collection of anaërobic cultures which now numbers 69 strains distributed among 15 clearly recognizable species and 4 strains as yet unidentified, we have observed that certain ones habitually form white crystalline products in the deep brain medium that is used for preserving the stock cultures; namely 3 strains of *B. bifermentans*, 4 of *B. centrosporogenes* (a new species shortly to be described for the first time), 1 of *B. histolyticus*, and especially an unplaced culture, herewith designated No. 106, that resembles *B. sporogenes* in certain properties but differs in its striking crystal formation. All of these are actively putrefactive anaërobes.

We have failed to observe such crystals in 6 strains of *B. Welchii*, 3 of *B. Novyi*, 2 of *B. butyricus*, 19 serologically homologous strains of *B. sporogenes*, 5 heterologous strains of *B. sporogenes*, 2 strains of *B. botulinus* Type A, 3 of *B. botulinus* Type B, 7 of *Vibrion septique*, 7 of *B. tetani*, 3 of *B. putrificus*, and 1 each of *B. Chauveauii*, *B. sphenoides*, *B. tertius*, and *B. tetanomorphus*. Of this list *B. sporogenes* and *B. botulinus* are actively putrefactive, *B. tetani* and *B. putrificus* less strikingly so.

References to crystals supposed on microscopic grounds to be tyrosine in cultures of putrefactive anaërobes are scattered through the literature, a review of which is reserved for a more detailed report. So far as we are aware, no one else has recovered tyrosin in a state of high purity from a pure culture of any single bacterium.

Our studies to date show that culture No. 106 produces crystals, macro- and microscopically resembling tyrosin, in ground meat, brain, salmon, milk and suspended casein mediums not containing fermentable carbohydrates, *i.e.*, monosaccharides in excess. The early stages of incubation are marked by clouding and vigorous gas production. The crystals begin to appear in 4–6 days at 37° C. along with a visible liquefaction of the protein as well as odorous evidence of putrefaction. Meat and brain mediums are blackened presumably owing to the precipitation of iron sulfide by the action of sulfuretted hydrogen upon the iron freed by proteolysis. Milk, salmon and casein mediums are not blackened, although sulfuretted hydrogen is produced, except upon the addition of iron ions, as by the inclusion of an iron nail. These facts fail to support a suggestion that the blackening of certain proteins by putrefactive anaërobes parallels the supposed action of a tyrosinase in transforming tyrosin into melanin in the animal body.

The recovery of the crystals in a pure form involves the removal of the water-soluble constituents by washing the partially digested culture mediums with cold water, extraction of the crystals by boiling water, with or without the addition of ammonia, followed by hot filtration to remove undissolved proteins, concentration of the filtrate by boiling, crystallization from the concentrated filtrate by cooling, removal of the crystals by cold filtration and repeated clarification with animal charcoal in boiling water alternated with crystallization in the cooled filtrate. Impurities soluble in cold water are removed by washing at each cold filtration and the crystals are dried after rinsing with 95 per cent. alcohol followed by ether.

Quantitative methods of extraction are yet to be devised; there is considerable loss at each step excepting in the treatment with ether. The sample displayed, about 0.8 gram, represents the purified product from several liters of culture.

The crystals are identified as tyrosine by their physical and chemical properties. To the naked eye they appear as snowy white flakes with a silken sheen, the individual needles barely visible. These flakes may be readily suspended in alcohol or distilled water; in the latter particularly the characteristic crystals may be seen with a hand lens as colorless double-pointed needles. When allowed to crystallize slowly from somewhat dilute hot water or ammonia solutions, they readily form the sheaf-like bundles so characteristic of tyrosin.

They are soluble in boiling water, N/10 ammonia, and dilute mineral acids, slightly soluble in dilute acetic acid and relatively insoluble in cold water, cold and hot absolute alcohol, ether, toluene, acetone, benzine, carbon disulphide, glycerine and chloroform. They are not decomposed in aqueous solution by heating in the Arnold sterilizer at 100° C. or in the autoclave at 15 lbs. pressure for 1 hour.

They give Pirie's, Hoffmann's and Denige's tests.[1]

The senior author is now engaged in perfecting the method of extraction and in studying the crystal formation of other anaërobes.

### 23 (1770)

## A method for the preparation of cystin.

By **CARL L. A. SCHMIDT.**

[*From the Department of Biochemistry and Pharmacology of the University of California, Berkeley, California.*]

A number of years ago Folin[2] described an improved method for the preparation of cystin which has come into general use. It is based on the fact that the solubility of cystin is a minimum in solutions possessing an acidity between $P_H$ 4–5. To obtain the optimum acidity for the precipitation of cystin, the HCl used to hydrolyze the protein is neutralized by the addition of sodium acetate. Although good yields of the amino acid are obtained by this method it nevertheless is not economical for the production of cystin in quantity since large amounts of relatively expensive materials are required. Neutralization of HCl with sodium acetate results in the simultaneous precipitation of humin which later necessitates the repeated use of large quantities of charcoal to effect its removal.

In the method described below the HCl is in large part recovered by vacuum distillation. Use is then made of commercial finishing lime to neutralize the remaining HCl, to precipitate the humin[1] and to hold the cystin in solution. The advantage in the use of lime lies in the fact that it is comparatively insoluble, gives a solution of low alkalinity, thus minimizing the destruction of cystin, and is cheap.

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[1] Hammerstein-Mandel, "A Text-book of Physiological Chemistry," J. Wiley & Sons, N. Y., 1912, p. 150.

[2] Folin, O., *J. Biol. Chem.*, 1910, viii, 9–10.

Human hair or wool which has been freed from oil by extraction with gasoline is hydrolyzed by heating at 100° C. with twice its weight of concentrated HCl. It requires about 12 hours to effect complete hydrolysis. The mixture must not be heated for any length of time beyond the point at which the biuret test is either negative or feebly positive since, as Van Slyke[2] has shown, cystin is destroyed during the process of hydrolyzing the protein. The greater part of the protein is removed by distilling in vacuo at a temperature between 60–70° C. and the original volume of the solution is restored by the addition of water. A thick aqueous suspension of commercial finishing lime is now slowly added, care being taken to avoid any considerable rise in temperature, until the mixture has acquired a chocolate color. It is then filtered by suction through a Buchner funnel and the residue washed a number of times with distilled water. The filtrate should be clear and possess a light brown color. Hydrochloric acid is now added to partially neutralize the alkaline solution and it is finally acidified by addition of acetic acid. On standing over night in the ice chest, sedimentation of the crude cystin takes place. This is filtered off and is dissolved in a minimum quantity of 5 per cent. HCl. The solution is decolorized by boiling for several minutes with a small quantity of charcoal which has been previously boiled with HCl to remove the calcium phosphate, and the cystin is precipitated by the addition of sodium acetate to the hot solution until a drop of the solution ceases to turn congo red paper blue. The mixture is filtered at once and the cystin is washed a number of times with hot water to completely remove the last traces of tyrosin. Typical hexagonal plates of cystin are obtained.

To compare the relative yields of cystin by the method of Folin and the above-described method, 1.3 kilos of human hair were hydrolyzed and divided into two equal parts. The yield of cystin isolated by the Folin method was 7 per cent., while the lime method gave a yield of 6.3 per cent.

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[1] Hanke, M. T., and Koessler, K. K., *J. Biol. Chem.*, 1920, xliii, 521–526.

[2] Van Slyke, D. D., *J. Biol. Chem.*, 1911, x, 38.

### 24 (1771)

## A globulin as the principal protein of the pecan nut: Its chemical and nutritive properties.

By **F. A. CAJORI** (by invitation).

[*From the Department of Chemistry, Stanford University, California.*]

Pecan meal, prepared by removing the oil from the whole shelled nut, was extracted with 10 per cent. sodium chloride solution. This extract containing the proteins of the meal was subjected to fractional precipitation with ammonium sulfate and fractional coagulation by heat. The results indicate that the large part of the protein of the pecan nut is a globulin. This globulin has been isolated and purified and the distribution of its nitrogen determined by the Van Slyke method. Large amounts of basic amino-acids were found to be present in this globulin. It gives a strongly positive test for tryptophane. In general the analysis agrees fairly well with the recently published results of Dowell and Menaul[1] on mixed pecan proteins.

Normal growth has been observed in young rats on diets in which the protein of the ration was derived from the pecan nut, indicating that this nut furnishes adequate quantities of those nitrogenous complexes necessary for growth. In order to render pecan nut diets suitable as rations for rats, it was found necessary to remove the outer layer of the nut since this layer contains large amounts of tannin. Previous failure to observe normal growth in rats on pecan nut diets may be ascribed to the injurious or distasteful effect of the tannins that were present in those diets, and not to an inadequacy of amino-acid yield of the protein of this nut.

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[1] Dowell, C. T., and Menaul, P., *J. Biol. Chem.*, 1921, xlvi, 437.

25 (1772)

## A modification of the Du Bois height-weight formula for surface areas of newborn infants.

By **HAROLD K. FABER** and **MARGARET S. MELCHER.**

[*From the Subdivision of Pediatrics, Stanford University Medical School, San Francisco, Cal.*]

In 1916 Sawyer, Stone and Du Bois announced a series of measurements by which the surface area of adults and children could be estimated with an average error of 1.3 per cent. In a subsequent paper of the same series (1916) by Du Bois and Du Bois, a new formula based on height and weight alone was presented which was stated to have an error of ± 5 per cent. This formula is as follows:

$$A = W^{.425} \times H^{.725} \times C,$$

or

$$\log A = \log W \times .425 + \log H \times .725 \times \log C.$$

The constant $C$ was found to be 71.84 (log 1.857).

It was not known whether the formula held for children under two years.

Since the first method is based on a separate estimation of the surfaces of the extremities, head and trunk there seems to be no reason why this method should not be applicable at any age. The height-weight formula should however apparently be checked for young infants.

In a series of 100 newborn babies, none over 12 days old, ranging in weight from 2,140 to 4,520 grams and in height from 45.2 to 56.9 cm., the surface area was measured by the Sawyer, Stone and Du Bois method and compared with the results obtained by the height-weight formula of Du Bois and Du Bois. Taking the former as the correct measure, we found that the latter showed a constant deviation below the former which averaged 191 sq. cm. or a mean error of − 8.6 per cent. Correcting the constant, it was found that the surface area could be computed in these infants by the height-weight formula with an average error of ± 2.5 per cent.

For newborn infants the corrected formula is as follows:

$$A = W^{.425} \times H^{.725} \times 78.50,$$

or

$$\log A = \log W \times .425 + \log H \times .725 + 1.895.$$

### 26 (1773)

## On the relation of blood-volume to the nutrition of the tissues.

## I. The effects of hemorrhage and intravenous injections of gum-saline on the response to the administration of a mixture of carbon dioxide and room air, and of room air alone.

By **ROBERT GESELL, CHARLES S. CAPP** and **FREDERICK S. FOOTE.**

[*From the Department of Physiology, University of California, Berkeley, California.*]

At the last meeting of the American Physiological Society some experiments were reported on the effects of hemorrhage on the response to a gradual reduction in the percentage of oxygen in the respired air. These experiments were performed on the normal unanesthetized dog connected by means of a mask with a rebreathing apparatus arranged to absorb the carbon dioxide of the expired air as the oxygen was consumed. The purpose of the experiments was to determine the detrimental effects of hemorrhage and the subsequent effects of replacing the lost blood with a gum-saline solution.

We reasoned that if a normal percentage of hemoglobin and a normal flow of blood are essential for a normal gaseous exchange, that the response of an animal to a reduction in the percentage of oxygen in the respired air would be altered by hemorrhage; and further that if the intravenous injection of gum-saline accelerated the volume-flow of blood out of proportion to the accompanying dilution, the reduced tolerance to low pressures of oxygen would be improved.

To our surprise we were unable to detect, with the methods employed, any decrease in tolerance after hemorrhage amounting to 3 per cent. of the body weight. We do not attempt to definitely explain these results as yet, but wish to point out a striking effect of hemorrhage. It invariably produced a quieting effect upon the dogs accompanied by a softness of muscle indicative of a marked reduction in muscular tonus. It is possible that the decreased demand for oxygen on the part of the tissues partly explains our data.

When carbon dioxide was allowed to accumulate in the rebreathing apparatus as the oxygen percentage of the respired air decreased, hemorrhage produced a definite decrease in tolerance to the combined changes in carbon dioxide and oxygen. But the restlessness of the animals under these conditions contraindicated an effort at quantitative results without anesthesia.

We have recently conducted a series of experiments on the dog under morphine-ether, and morphine-urethane anesthesia. In these experiments we compared the ventilation during the administration of a mixture of carbon dioxide and room air with that of room air—first with a normal blood volume, second after hemorrhage, and third after the replacement of the blood with gum-saline. Consistent results were obtained in 10 experiments. The administration of a 5 per cent. mixture of carbon dioxide in room air invariably increased the ventilation over that of room air alone. The increase in ventilation was markedly augmented by hemorrhage. In some cases the augmentation was excessive. Subsequent injections of gum-saline reduced the response almost to that obtaining before hemorrhage. Striking augmentation of response to carbon dioxide mixtures resulted from hemorrhages amounting to 1 per cent. of the body weight and less. In some experiments changes in respiration were associated with the administration of carbon dioxide alone. In other experiments the ventilation of room air was increased by hemorrhage, and decreased by injection as well.

These experiments, we believe, demonstrate that hemorrhage amounting to 1 per cent. of the body weight or less may, under the conditions employed in these experiments, produce a detrimental effect upon the volume flow of blood, and that the injection of an inert solution improves the circulation in such a way as to enhance the gaseous exchange.

27 (1774)

## On the relation of blood-volume to the nutrition of the tissues.

### II. The effects of hemorrhage and subsequent injections of gum-saline upon the volume-flow of blood through the striated muscle of the dog.

By ROBERT GESELL.

[*From the Department of Physiology, University of California, Berkeley, California.*]

The effects of blood-volume upon the volume-flow of blood were studied by means of the drop method. While in some animals hemorrhage amounting to 1 per cent. of the body weight had a relatively small effect, in many animals the same or smaller hemorrhage markedly decreased the volume-flow of blood. Subsequent injections of a 6 per cent. suspension of gum in a 0.9 per cent. sodium chloride solution augmented the flow out of all proportion to the dilution entailed.

28 (1775)

## On the relation of blood-volume to the nutrition of the tissues.

### III. The effects of hemorrhage and subsequent injection of gum-saline upon the response of the sartorius muscle of the dog to rapid electrical stimulation.

By ROBERT GESELL.

[*From the Department of Physiology, University of California, Berkeley, California.*]

By comparing fatigue curves elicited by equal periods of stimulation of the sartorius muscle, we found that hemorrhage had a detrimental effect upon the endurance of the muscle, that hemorrhage amounting to 1 per cent. of the body weight or less may decrease the response of the muscle to stimulation. Subsequent injection of gum-saline decidedly improved the response of the muscle to further stimulation.

### 29 (1776)

## On the relation of blood-volume to the nutrition of the tissues.

## IV. The effects of hemorrhage and subsequent injection of gum-saline on total oxygen consumption.

By ROBERT GESELL, CHARLES S. CAPP and FREDERICK S. FOOTE.

[*From the Department of Physiology, University of California, Berkeley, California.*]

The effects of progressive hemorrhage were studied on the dog under morphine-urethane anesthesia. We found that the greater the hemorrhage the greater the reduction in the amount of oxygen consumed and that a hemorrhage amounting to 1/2 per cent. of the body weight may elicit a decided reduction. Subsequent injection of gum-saline, bringing the blood volume back to normal, increased the amount of oxygen consumed. The amount of oxygen consumed immediately after an injection was greater than the consumption a few minutes later. We believe this, along with the decreased amount of oxygen consumed, points to an oxygen hunger during a period of decreased blood-volume. The results here reported are in agreement with those recently published by Doi.[1]

### 30 (1777)

## A comparison of the waves of blood pressure produced by slow and by rapid breathing.

By ROBERT TROTTER, PHILIP EDSON and ROBERT GESELL.

[*From the Department of Physiology, University of California, Berkeley, California.*]

The effects of rapid breathing were compared with those of more normal breathing upon the systolic blood pressure in man. Supplementary data were also obtained on the dog and cat.

For the well-known changes of blood pressure that occur during a single respiration, and which are more or less synchronous with the changing respiratory phases, we have proposed the name of simple cardio-respiratory waves to distinguish them from those waves produced by rapid breathing.

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[1] Doi, Y., *Journal of Physiology*, 1921, lv, 249.

The oscillations of pressure elicited during rapid breathing by the interference method we have designated as cardio-respiratory interference waves.

The most striking difference in the respiratory relations of the simple and interference waves is that in the simple waves the blood pressure changes are complete within the period of a *single* respiration, while in the interference waves the gamut of the blood pressure changes is run through in the interval of *several* respirations.

The production of interference waves of blood pressure is dependent upon the establishment of cardio-respiratory cycles in which the number of respirations is greater by one or lesser by one than the number of heart beats making up the waves and occurring in the same time interval.

When these conditions are fulfilled we may conceive of the heart beats as moving through respiration, the direction of the movement being determined by the relative rates of the heart and respiration, that is, whether the respiratory rate is slower or faster than the heart rate. A cardio-respiratory cycle is complete when two beats (the first and last of the interference wave) fall at approximately the same time in respiration.

Whereas in the simple respiratory waves we found the highest pressure to be associated with approximately the beginning of inspiration, in the cardio-respiratory interference waves we found the highest pressure to obtain at approximately the beginning of expiration.

Without definitely assigning the responsibility for the production of interference waves to any particular respiratory factor, we are inclined to favor the hypothesis that they are primarily due to the changing intra-thoracic pressure accompanying respiration.

It is possible by breathing slightly slower or slightly faster than half the heart rate to produce double interference waves of blood pressure, that is, under these conditions, two waves of blood pressure may be in progress simultaneously. Each of the double waves is formed by alternate heart beats, one being made up of the even numbered and the other of the odd numbered beats. Double cardio-respiratory interference waves are to be explained in the same manner as the single waves.

The results of these experiments on the dog and cat are in agreement with those obtained in man.

Cardio-respiratory interference waves particularly of the double type with alternating beats occur simultaneously in sacrifice experiments in dogs and cats. We therefore point to our work as occasionally explaining pulsus-alternans and blood pressure waves of the third order.

## ABSTRACTS OF THE COMMUNICATIONS, MINNESOTA BRANCH.

### First meeting.

*Minneapolis, Minnesota, October 12, 1921.*

31 (1778)

### Experimental rickets.

By **J. F. McCLENDON** and **HARRY BAUGUESS** (by invitation).

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minnesota.*]

In a large number of experiments on feeding albino rats in which white wheat flour was used as the main part of the ration, bone abnormalities were absent or transitory. Since Sherman showed that rickets could be inhibited by simply adding phosphate to the diet we concluded that the normal condition of the bones of our rats was due to the phosphoric acid content of the casein used in the diet. After substituting casein by lactalbumin or edestin, bone abnormalities appeared in one hundred per cent. of our rats. If casein was fed to the extent of 6 per cent. of the ration bone abnormalities were reduced and apparently the disturbance was transitory, since the bones became hard and cast dense shadows with the x-rays without changing the diet, yet some of the deformities were preserved. With a basic ration of white flour containing 6 per cent. sea salt, the addition of edestin or lactalbumin to improve the protein did not in any way decrease the abnormalities. The edestin carries vitamine B and if wheat germ extract is added to the lactalbumin in order to furnish vitamine B, the abnormalities still persist. The addition of spinach up to 5 per cent. of the ration did not decrease the abnormalities, whereas 0.2 per cent. furnished sufficient vitamine A to keep the animal alive for 3 or more months. The addition of 0.5 gram of butter fat per day did not lessen the abnormalities. In order to determine the growth of the bones x-ray plates were made by photographing a large number of animals on the same plate and comparing the density of the shadows of the bones. In addition, determinations of calcium intake and output and calculations of the calcium retention were made. Considerable individual variation was found, but when long metabolism periods (about a week) were used and all our animals with abnormal bones were averaged we found that rats which were weaned and placed on the diet at the age of twenty-one days and left on this diet at least three days before commencing the metabolism study showed a retention of 2.7 milligrams of calcium per rat per day for the first two weeks, 1.7 for the third week and 1.5 for the fourth week. In some individual rats shortly before death we obtained negative calcium balances. It would indicate, therefore, that the disturbance of calcium metabolism is increasing in severity from the first to the fourth week. We do not know the average calcium retention of the normal rat but we assume that it is close to 5 milligrams per day for the ages corresponding to our rats. This is based on calcium content of whole rats. It seems probable, therefore, that calcium retention can be used throughout the course of the disorder as an index of the severity of the disease, provided adequate methods are used to determine the calcium retention. In order to avoid errors due to transfer of the excreta we have made round cages with quarter-inch-mesh wire screen bottoms that sit in silica dishes six inches in diameter and have two bird-feed cups attached, one for water and one for food. Any food spilled from the container into the dish does not cause an error because it does not affect the difference between the intake and the outgo of calcium. At the end of the metabolism period the cage is lifted from the dish and the excreta ashed in the dish. In order to separate the calcium from the dissolved ash as calcium oxalate we have used a bromphenol blue as an indicator for hydrogen ion concentration. If the solution which is acid is neutralized until it reaches $P_H = 4$ the calcium oxalate will not be appreciably soluble and yet no calcium will precipitate as phosphate. In case of adding too much alkali it is best to use acetic acid to bring the solution back to $P_H = 4$, because the danger of overstepping the end point is very small, since the mixture of sodium acetate and acetic acid can vary considerably in composition with little change in the hydrogen ion concentration. This is McCrudden's method in principle, but the indicator makes us more certain of the $P_H$. The calcium oxalate was titrated with potassium permanganate.

We do not wish to discuss the diagnosis of rickets, although our animals showed the same appearances as those described in the papers of Sherman, McCollum and Hess. Dr. C. M. Jackson has very kindly offered to work out the morphological changes in great detail and publish them so that they will be available. From a practical standpoint, however, types of non-rachitic osteoporosis caused by calcium deficiency do not seem to be very common among human beings. Bone abnormalities which are possible on human diets at present in use deserve considerable study whether they are called rickets or not and we use the word rickets merely for convenience. Some of our rats getting more phosphoric acid than that contained in the wheat flour seemed to recover from the disturbance of metabolism just as infants may recover from rickets, with the reservation that the diets of our rats were unchanged in percentage-composition and only changed in the quantity eaten per day, whereas very little exact data is to be had on the diets of human beings. In case the abnormalities are not great enough to cause permanent deformities we do not know of any means of detecting the previous history of rickets without diagnosing it at the time of its occurrence. The x-rays and calcium balances, however, may be used for diagnostic purposes on rats without killing the animals. Rats die easily under ether and it is difficult to get them absolutely quiet without danger of killing them unless they are held mechanically. They may be stretched out by tying their feet to a stiff ring of suitable size after slightly etherizing them.

## 32 (1779)

## Rapid determination of surface tension.

By **ROBERT G. GREEN** (by invitation).

[*From the Department of Bacteriology, University of Minnesota, Minneapolis, Minn.*]

An apparatus was demonstrated by means of which the surface tension of a liquid is rapidly determined by the drop-weight method. From one to six drops of the liquid to be measured is required. The apparatus consists essentially of a delicate torsion wire balance and an adjustable scale on which the surface tension is read in dynes per centimeter.

## 33 (1780)

## The influence of the surface tension of the culture medium on bacterial growth.

By **W. P. LARSON.**

[*From the Department of Bacteriology, University of Minnesota, Minneapolis, Minn.*]

Pellicle-forming bacteria such as the *B. tuberculosis*, *B. subtilis* and others of that group which habitually grow upon the surface of liquid medium, will grow throughout the body of the medium by depressing its surface tension from 59 dynes, the S. tension of ordinary broth, to 40–45 dynes. By analogy with the floating needle experiment it may be assumed that when the pellicle-forming bacteria are properly wetted they no longer grow upon the surface of the medium but throughout the body of the broth or even at the bottom of the flask.

The further observation has been made that the *B. subtilis* and *B. anthracis*, when grown in media of low S. tension, finally become asporogenous. Cultures of *B. anthracis* grown under such conditions and sterilized by heat at 60° for 30 minutes protect guinea pigs. The enhanced wetting of the bacteria brought about by the addition of soap probably creates better nutritive conditions which cause the organisms to grow without forming spores. Castor oil soap when in aqueous solution is perfectly clear, does not hydrolyze as readily as most other soaps, and has therefore been used extensively in our experiments. It is more toxic to some bacteria than potassium or sodium stearate. This is probably due to the fact that castor oil soap is dialyzable and probably dialyzes into the cell and disturbs the salt balance by precipitating the calcium, magnesium and salts of the heavy metals.

Bacteria such as the pneumococcus and streptococcus will not grow on low tension media, while the organisms which inhabit the intestinal tract grow abundantly on media of low tension. This is not surprising since the contents of the intestines have a low S. tension due to the presence of bile, soaps and other S. tension depressants. It is well known that many of the intestinal bacteria when inoculated in broth grow near the surface of the medium. This is particularly true of the cholera vibrio. This selective localization is probably due to the fact that the S. tension reducing substances concentrate at the surface of the medium thus creating a favorable environment for these bacteria. Incidentally it may be pointed out that the bacteria which grow well in low tension media are better antigens than the streptococcus, pneumococcus and others which refuse to grow under such conditions.

## 34 (1781)

## A micro-Winkler method for the quantitative determination of dissolved oxygen.

By E. J. LUND.

[*From the Department of Animal Biology, University of Minnesota, Minneapolis, Minn.*]

Winkler's method for quantitative determination of dissolved oxygen may be applied to 10 c.c. or even 5 c.c. samples of water in the following way. One tenth of a cubic centimeter of each of the two solutions $MnCl_2$ and $NaOH - KI$ are added from 1 c.c. burettes graduated to 0.1 c.c. or less. The thiosulfate solution of the usual concentration is diluted to ten times its volume. The iodine is titrated in a tall dish using a 5 c.c. burette. The end joint is just as definite as that in the ordinary procedure. The percentage error is also the same, about 1 per cent. The distinct advantage in the micro method lies in the possibility of greatly shortening the duration of the tests, thus making it possible to follow the time course of respiratory exchange over relatively short periods of time. Owing to the small volumes used, temperature adjustment is rapid.

With good manipulation the maximum error is less than 0.005 c.c. of $O_2$ gas. The method is being used in studies on oxygen consumption by small organisms such as protozoa, eggs and certain kinds of tissues.

### 35 (1782)

## Does the introduction of an ethoxy group into aromatic compounds increase their bactericidal action upon the pneumococcus and the gonococcus? [1]

By **ARTHUR D. HIRSCHFELDER** and **L. J. PANKOW.**

[*From the Department of Pharmacology of the University of Minnesota, Minneapolis, Minn.*]

Morgenroth and his collaborators have shown that when an ethoxy group is substituted for the methoxy group in quinine derivatives and ethylhydrocuprein is produced, the substance takes on markedly increased pneumococcicidal action in vitro and in vivo. Solis Cohen, Kolmer and Heist found that ethylhydrocuprein hydrochloride was from eight to twenty times as strong an antiseptic for the pneumococcus as quinine hydrochloride. Morgenroth and Levy had shown that no such difference between quinine and ethyl hydrocuprein could be observed in the case of the streptococcus. We find that when cultures of gonococcus are exposed to starch bouillon containing quinine hydrochloride or ethylhydrocuprein in 1/10,000 dilution and then transferred to plates of rabbit's blood agar, growth occurs if the exposure to the drug has lasted only ten minutes but the bacteria are killed if the exposure has lasted thirty minutes. Ethylhydrocuprein has therefore no specific action against the gonococcus. However, as ethylhydrocuprein is too toxic for successful use in the chemotherapy of lobar pnuemonia, substances which are less toxic must be sought for.

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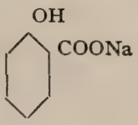
[1] The investigations recorded in this paper were rendered possible by a grant of funds granted by the United States Interdepartmental Social Hygiene Board, for the discovery of better medical measures for the prevention and treatment of the venereal diseases.

The first question to be determined is whether pneumococcicidal properties are common to ethoxy compounds in general or whether this property is peculiar to ethylhydrocuprein and its closely related compounds. We have accordingly tested the bactericidal action of the ethyl ethers of various aromatic compounds, and compared them with the corresponding hydroxy compounds. In making the tests a suspension of pneumococcus type I. from the Rockefeller Institute was suspended for the desired interval in a broth solution of 0.9 per cent. NaCl containing the substance whose action was to be determined; and after the desired interval a loopful of this stroked across a rabbit's blood agar plate and incubated 24 hours. In the experiments with the gonococcus a strain was used which had been isolated from a case of typical anterior gonorrhoeal urethritis in the Outpatient Service of the Genito Urinary Division of the University of Minnesota, and which had been grown in Vedder's starch bouillon and on starch bouillon agar and rabbits' blood agar. Tests were made in the same way as for pneumococcus except that the drug was mixed with starch bouillon. The cultures were then transferred to rabbits' blood agar and incubated 24 hours.

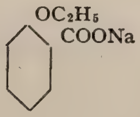
The following substances were tested, + indicating growth of the cocci after being exposed to the drug for the period indicated,—indicating that the cocci did not grow.

1. Sodium salycilate



5% the Pneumococcus + after 10 min.
− after 60 min.

2. Sodium ethylsalicylate[1]



5% Pneumo + after 60 min.

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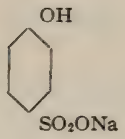
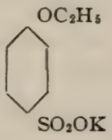
[1] Substances Nos. 2, 4, 10, 11, 12, 13 were prepared by Mr. Merrill C. Hart, chemical assistant in the department of pharmacology, University of Minnesota, and No. 9 was kindly furnished by Prof. Roger Adams, University of Illinois, to both of whom we extend our thanks.

3. Sodium phenolsulphonate



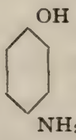
5% Pneumo + after 2 hours.
1% Gono + after 30 min.

4. Potassium phenetolsulphonate


$OC_2H_5$
$SO_2OK$

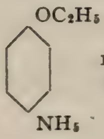
5% Pneumo + after 2 hours.
1% Gono + after 30 min.

5. **Para** aminophenol



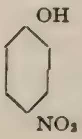
1% Pneumo + sometimes — sometimes after 30 min.
1 : 1000 Gono — in 10 min.

6. Para phenetidin



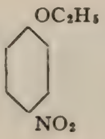
1% Pneumo + sometimes — sometimes after 30 min.
1% Gono — after 10 min.

7. Para nitrophenol



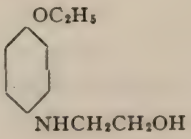
1% Pneumo + after 10 min. — after 30 min.
1 : 500 Gono + after 10 min. — after 30 min.

8. Para nitrophenetol



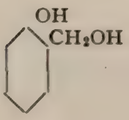
1 : 1000 (sat. sol.) Pneumo + after 60 min.
1 : 1000 Gono + after 60 min.

9. Phenetidinethylalcohol


$OC_2H_5$
$NHCH_2CH_2OH$

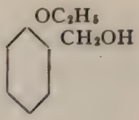
Pneumo + after 30 min.
1 : 250
Gono + after 30 min.

10. Saligenin



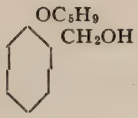
2% (in serum) Pneumo — after 60 min.
2% Gono + after 30 min.
— after 60 min.

11. Saligenin ethyl ether


$OC_2H_5$
$CH_2OH$

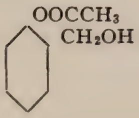
Sat. sol. in 0.9% NaCl (about 1 : 10,000).
Pneumo. *Results vary.*
8 repetitions — after 5 min.
16 repetitions + after 30 min.

12. Saligenin iso amylether



Sat. sol. less than 1: 10000.
Pneumo — after 5 min.
Gono — after 10 min.
*Staphylococcus* and *Bacillus coli* both + after 30 min.

13. Acetyl saligenin



1: 200 Pneumo — after 5 min.
Gono — after 10 min.

From the above-recorded experiments it is evident that in the simpler aromatic substances, predominantly water-soluble like sodium phenolsulphonate and salicylate or predominantly lipoid-soluble like para amino phenol and para nitrophenol, whether nitrogen-free or containing nitrogen, the introduction of an ethyl group upon the ring does not confer pneumococcicidal or gonococcicidal powers. Whenever any difference is noted the hydroxy compound is a somewhat stronger antiseptic than the ethoxy. There is therefore no analogy in this regard between the simpler aromatic compounds and the quinine derivatives.

# SCIENTIFIC PROCEEDINGS.

## ABSTRACTS OF COMMUNICATIONS.

### One hundred eighteenth meeting.

*New York Post-Graduate Medical School, November 16, 1921.*
*President Wallace in the chair.*

### 36 (1783)

### Alcohol and white rats: a study of fertility.

By E. CARLETON MACDOWELL.

[*From the Station for Experimental Evolution, Cold Spring Harbor, N. Y.*]

This paper deals with the effect of alcohol fumes upon the size and number of litters produced by white rats and their descendants. Details of the administration of the alcohol have been published.[1] The treatment was given from the age of 28 days through the lives of the rats, with the exception of the females on the 28 days following the birth of a litter. After mild initial doses, each rat was left in the fume tank each day until it was completely anesthetized. Brothers and sisters of the treated males and females were used as controls. The matings were all between treated males and treated females or their descendants, and between the controls. Each group of test matings in each generation had its own control group raised at the same time and under the same conditions of environment. The data came from four main groups of rats: those treated, their treated children, their untreated children, and their untreated grand children from the untreated children.

*Size of Litters.*—The average size of all the litters produced by the treated rats was 10 per cent. less than the control average. Nine pairs of treated offspring from these treated rats gave litters that were 10.3 per cent. smaller than their control litters. Ten pairs of untreated rats from the treated parents gave litters that were 11.2 per cent. smaller than the controls. And eleven pairs of untreated rats from untreated parents and treated grandparents gave litters that were 13.3 per cent. smaller than their control litters. So there appears to be a reduction in the average litter size in each generation that is about ten per cent. of the respective controls. However, in no case is the reduction statistically significant. This is probably due to the small size of each sample, since the combination of all the generations gives a difference that is 3.6 times its probable error and so is to be considered significant. Alcohol appears to have caused a modification in litter size that persists for two generations after the original treatment and is not increased by a second generation of treatment. This is the result to be expected from a definite germinal modification.

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[1] MacDowell and Vicari, *Jour. Exp. Zoöl.*, 1921, xxxiii, 209.

*Number of Litters.*—The numbers of litters are based upon the production during equal periods of the test pairs and their own particular control pairs. In some cases the period was longer than in others, but opportunities for the tests and controls to produce litters were equal in each case and therefore equal in the totals. The number of litters is, accordingly, purely relative. 44 treated pairs produced 32 litters, whereas on the basis of their controls 91 litters were expected. This was a reduction of 65 per cent. ± 3.37 which is 19.2 times its probable error and so, significant beyond all question. Treated rats from treated parents produced 14 litters when 22 were expected on the basis of their controls. This is a reduction of 35 per cent. ± 6.91 and is 5 times its probable error. Untreated rats from treated parents produced 33 per cent. ± 8.20 *more* litters than the controls, and the untreated rats from untreated parents and treated grandparents 55 per cent. ± 8.4 *more* litters than the expectation. Both these differences are fully significant. The number of litters was strongly reduced when the rats themselves were treated, but, just as soon as the alcohol was further away, the number of litters at once increased and the test animals produced significantly more litters than their controls. The obvious interpretation of this result is that alcohol has acted as a selective agent by preventing those females from having litters that bore weaker determinants for the production of litters. This accounts for the apparently odd fact that two generations of treatment made less difference than a single generation of treatment. The offspring of treated animals are a selected lot. Genetically they have higher litter-producing powers than the controls; when alcohol is given to them it causes a reduction in the number of their litters, but this reduction is half as great as the reduction caused by the treatment of their parents which were genetically equal to the controls. The alcohol has sorted out differences already present.

This is a very different result from that given by litter size, which demands the assumption of alcohol modifications. It is to be concluded, then, that alcohol works upon the size of litters and the number of litters through different channels. This leads to two generalizations: first, that fertility is a complicated character whose different measures are not all manifestations of the same factors; second, that the action of alcohol upon animals is very complicated; it may act through different channels and in different ways, so that the end results in any special case are due to the interaction of different tendencies. Students of experimental alcoholism must recognize the complex nature of their problem, and, leaving behind the familiar method of generalizing from end results, focus attention upon the problem of the channels through which alcohol may work.

### 37 (1784)

## Experiments with B. enteritidis (murium)[1] on normal and immune mice.

By LESLIE T. WEBSTER.

[*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*]

These experiments were undertaken to ascertain varieties and degrees of resistance of normal and immune mice to fixed doses of *B. enteritidis* (*murium*).

1. If live cultures of this organism are injected intrapleurally or intraperitoneally into normal mice, there occurs an initial lag in the rate of bacterial multiplication lasting a few hours followed by a rapid and continued acceleration of growth until the death of the animal.

If live cultures of this organism are given *per os* to normal mice, there occurs an incubation period of 5–6 days, after which the animal usually develops symptoms of disease and succumbs. A small percentage of mice, however, prove refractory to infection by this route.

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[1] A serological and cultural description of this organism will appear in the *Journal of Experimental Medicine.*

2. If live cultures of this organism are injected intrapleurally or intraperitoneally into mice previously "vaccinated" intrapleurally or intraperitoneally, they are partially destroyed and held in check by the protective mechanisms of the animal body for two or three days. Subsequently, the rate of bacterial multiplication increases gradually until the death of the animal. The partial protection following this type of vaccination is entirely of a general nature; no evidence of a "local immunity" has been obtained.

Mice given 1, 2, or 3 subcutaneous doses of this organism vaccine show a similar relative increase in resistance to the subsequent injection of live organisms *per os* as intraperitoneally.

3. Feeding mice live or killed cultures of this organism induces a definite protection against subsequent intrastomachal and intraperitoneal injections of live organisms. The immunity developed in this way is also of a "general" as opposed to a "local" nature.

### 38 (1785)

## Therapeutic application of Bacillus acidophilus.

By **LEO F. RETTGER** and **HARRY A. CHEPLIN.**

[*From the Bacteriological Department, Yale University, New Haven, Conn.*]

In previous communications to the Society (1920 and 1921) we stated that *Bacillus acidophilus* may be implanted in the human intestine by the oral administration of (1) minimum quantities of lactose or dextrin, (2) whey broth cultures of *B. acidophilus*, or (3) a combination of lactose and the *acidophilus* culture in which the amounts of each are cut in half. Early in 1921 the milk culture of this aciduric organism was substituted for the lactose- and whey broth cultures and subsequent implantation experiments have been carried on with the *acidophilus* milk.

In the work on pathological cases we received the friendly coöperation of practicing physicians, who not only supplied us with many of the most interesting subjects, but who furnished us with a history of the individual cases, and in two instances with X-ray photographs. The subjects comprising the first group under observation may be divided for convenience into the following classes:

| | |
|---|---|
| Chronic constipation with the symptoms of so-called autointoxication and other pathological conditions, some of them acute | 20 |
| Chronic diarrhea following an attack of bacillary dysentery | 2 |
| Colitis, at times bloody, and more or less mucous | 2 |
| Sprue | 2 |
| Dermatitis (eczema) | 3 |

These 29 cases are exclusive of those which have come under our observation within the past two months; nor do they include those which have been and are being studied in other institutions through our coöperation. Brief reference will be made to only a few of these 29 cases.

The subjects were instructed to bring to the laboratory one or two specimens of stool before taking the *acidophilus* treatment, and for a while daily samples, when procurable, after the first use of the *acidophilus* milk or of the milk plus stated amounts of lactose. Bacteriological examinations were made of these specimens and the results correlated with the clinical findings. Persons who had been afflicted with chronic constipation usually received one quart of the *acidophilus* milk plus 100 grams of lactose daily, the powdered lactose being added to the milk in the flask and the contents thoroughly shaken. The subjects were instructed to take the daily supply in three equal portions, and as nearly as possible two hours before or after meals. This schedule was at times varied to suit the particular needs of the cases. There were no restrictions as to diet, but the subjects were urged to refrain from the use of food which by experience or training they knew to be harmful.

In all of the diarrheal cases (including colitis and sprue) the treatment consisted in the daily administration of from 500 to 1,000 c.c. of the *acidophilus* milk without added milk sugar. The milk was well tolerated by the patients.

## CHRONIC CONSTIPATION.

The first two cases, which had a long history of most obstinate constipation and in whom the symptoms usually accompanying such condition were of the most aggravated type, responded within the course of less than a week to the use of one quart of *acidophilus* milk daily without any added lactose. A very close correlation between the clinical and bacteriological results was established. Two other subjects who for many years had had marked enteroptosis and a condition approaching at times intestinal stasis yielded readily to the administration of one quart of the milk plus 100 grams of lactose, and after the first few days required only 500 c.c. of the milk with as little as 25 to 50 grams of added milk sugar daily. A fifth subject, however, obtained little relief from the treatment until at least two weeks after the first application. Within a month his condition was greatly improved. Two other subjects acted somewhat similarly, and another required a quart of the *acidophilus* milk plus 150 grams of sugar daily before a satisfactory response was obtained. In every instance sufficient relief was obtained to enable the patient to dispense, for the time at least, with the use of a cathartic, and a general, though at times slow, improvement of the patient's condition was manifested. More recently, however, one subject failed to react appreciably to the treatment, even when the amount of sugar was greatly increased. We shall expect to find other exceptions.

### CHRONIC DIARRHEA.

One of the two cases of chronic diarrhea following bacillary dysentery responded readily to the use of one quart of *acidophilus* milk daily. He was a Bohemian, male, forty-two years old, and had, with brief intermissions, suffered from the condition since 1907. The other was a returned Red Cross nurse who had contracted bacillary dysentery while on duty in the Balkans during the recent war. She obtained almost immediate relief, but experienced cycles of increasing and decreasing disturbance. She continued taking 500 c.c. of *acidophilus* milk daily for at least three months. During this time the more serious phase of the cycle became less and less acute until it became barely noticeable and the subject considered herself practically recovered. During the past two months she has reported from time to time, and consumed about one pint of the milk per week. She has had no recurrence of the diarrhea, and is able to devote her entire time to her occupation.

### COLITIS.

The two cases of colitis were of the acute type. One was uncomplicated, but had a long history of intestinal disturbance. The case responded to the treatment slowly and at the end of about four weeks all of the symptoms of colitis, which at times had been bloody, disappeared. He continued in apparently good health for about two months when he experienced a slight reversion.

The second case gave every evidence of improvement when, owing to a return of a serious nephritic condition, he required special hospital treatment and was compelled to discontinue the use of the *acidophilus* milk.

### SPRUE.

Both of the subjects had contracted sprue while in China. The one, whose case was less severe than the other, took one quart of the milk for six weeks, during which time the feces changed completely from the clay-colored, soft and extremely offensive to the yellow, almost formed and odorless type. The gas disappeared from the colon, and the subject appeared in every way to approach normalcy.

The second case was extremely acute, with the various advanced symptoms, including tetany. He was very emaciated and subject to abdominal pains and gaseous distention. There was a history of tubercular infection also. He seemed from the time of beginning the treatment to show a gradual improvement. The pains became less severe and the tetany disappeared for a time. He was able after several weeks to leave his room and to take short automobile excursions. He gained at least four pounds in weight, and seemed to be on the way to recovery when he experienced a relapse and died.

### DERMATITIS.

All of the three cases of dermatitis were those of eczema. One responded completely to the treatment, though it required about a month to bring about the first indications of a clearing up of the face, which for many years had been subject to a form of eczema that had caused considerable disfigurement. He required a quart of *acidophilus* milk plus 100 grams of lactose daily to bring about the desired implantation in the intestine. After two months following the beginning of the treatment all evidence of eczema had disappeared.

The second case showed some evidence of responding when the treatment had to be discontinued owing to an infection which developed in the hands which had been the parts mostly affected by the eczema, and to necessary medical treatment of the infected hands. The third subject was slow to react to the use of the milk and lactose and his condition showed little, if any, improvement.

The principles of the *acidophilus* treatment have been clearly set forth in different publications from the laboratory, and it is only when these principles are adhered to that favorable results should be expected. The ingestion of relatively small numbers of the bacilli should not be expected to lead to implantation and bodily improvement. Futhermore, the viability of the organism must be preserved in its preparation for therapeutic purposes. Finally, it should be understood that the *B. acidophilus* is not a panacea for all ills.

### 39 (1786)

## Growth and reproduction upon simplified food supply. II. Influence of food upon mother and young during the lactation period.

By **H. C. SHERMAN** and **MARIE MUHLFELD.**

[*From the Department of Chemistry, Columbia University, New York City.*]

Breeding rats were fed upon diets containing respectively one sixth whole milk powder to five sixths ground whole wheat or one third whole milk powder to two thirds ground whole wheat. Young were successfully reared on both diets and both would be regarded as adequate for growth, reproduction and successful suckling of the second generation. The larger proportion of milk in the second diet resulted in the following evidences of improved nutrition: (1) Increase in the number of young produced. (2) Increase in the percentage (and therefore also in the number) of young successfully suckled. (3) Better maintenance of the body weight by the mother while suckling the young. (4) Higher average weight of young at a standard weaning age of four weeks.

(5) More economical utilization of the calories of food consumed (as well as of the body material of the mother) in the rearing of the young to weaning age.

## 40 (1787)

## Some phases of the disinfection theory.

By **BARNETT COHEN.**

[*From the Hygienic Laboratory, Washington, D. C.*]

*Bact. typhosum* and *Bact. coli* (*communis*) were suspended in distilled water, tap water, and M/500 buffer solutions, respectively, at constant temperature levels (0°, 10°, 20°, 30° C.); and the numbers of survivors were determined by means of decimal dilutions upon agar plates. The conditions imposed (moderate H-ion concentrations at moderate temperatures) permitted a closer study of the disinfection process than has been usually possible.

It was found, of course, that *coli* was relatively more resistant than *typhosum*, but this greater resistance (at $P_H$ 3.5) decreased as the temperature level rose. At 0° C., *coli* was 67 times more resistant, and at 30° C. it was only 8 times more resistant than *typhosum*. There was a high inconstancy in results between duplicate tests carried out in tap or distilled water. This inconstancy could at times be correlated with comparatively insignificant fluctuations in $P_H$ of the water. When very dilute (M/500) Clark and Lubs buffers were used, this variability disappeared very largely.

At 20° C., *Bact. typhosum* possesses the greatest tolerance within a narrow zone delimited by $P_H$ 5.0 and 6.4. A slight increase in acidity beyond the zone results in conditions of maximum mortality. For *Bact. coli* the zone is wider and centered about neutrality. Cohen and Clark[1] found that the $P_H$ optima for growth and fermentation of bacteria may be different. It is now found that the optimum for tolerance may also be distinct.

The logarithmic decline in numbers of bacteria may be modified by suitably chosen conditions. This applies also to some monomolecular chemical reactions. The logarithmic course in either case is merely a statistical integration and gives no information as to the mechanism of chemical decomposition or of bacterial disinfection. They both illustrate the operation of the law of mass action.

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[1] Cohen, B., and Clark, W. M., *Jour. Bact.*, 1919, iv, 409.

The extended report of these experiments will be published soon in the *Journal of Bacteriology.*

### 41 (1788)

## A modified Hellige colorimeter for the comparison of solutions containing two colors.

By VICTOR C. MYERS.

[*From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York City.*]

By introducing an additional standard wedge in the Hellige colorimeter it has been found possible to greatly extend the usefulness of this instrument. With the instrument thus modified, the determination of the hydrogen ion concentration may be very quickly and accurately made by the colorimetric method, since with the two wedges it is possible to obtain all the shades of color from the acid to the alkaline side of the indicator. To aid in reading, the instrument has been provided with an eyepiece with lens.

With the aid of Sørensen's phosphate solutions (standards of $P_H$ 5.2 and 7.4 for brom cresol purple and standards of $P_H$ 6.4 and 8.4 for phenol red) it is possible to cover the range of $P_H$ 5.3 to 8.3 with an accuracy in reading of ± $P_H$ 0.02 to 0.04. This covers the most used range in the determination of the hydrogen ion concentration of urine, blood and bacteriological culture media. The phenol red standards also serve excellently for the Marriott alveolar carbon dioxide test.

It is a matter of common observation that it is rarely possible to obtain an exact color match with the standard in the phenolsulphonephthalein renal function test. By using the acid (yellow) phenol red standard in conjunction with the 'phthalein standard it is always possible to obtain an exact color match. If desired correction may be made for the rather small error introduced by the "off" color.

With thymol blue standards the determination of the $P_H$ of gastric juice may be very easily and quickly made.

Other uses of this instrument are being considered.

### 42 (1789)

## The effect of pancreatic rennet on blood coagulation.

By **ALBERT A. EPSTEIN** and **NATHAN ROSENTHAL.**

[*From the Department of Physiological Chemistry, Mt. Sinai Hospital, N. Y. City.*]

At the last meeting of this society, one of us[1] presented certain observations on pancreatic rennet. Particular stress was laid on the state in which the rennet probably exists in pancreatic extract, and its intimate chemical association with trypsin. In speaking therefore of the pancreatic rennet we wish it understood that it is the rennet-trypsin unit and not the rennet alone that we are dealing with. Mention was also made of the absolute dependence of the milk coagulating function of rennet on the calcium ion.

The theoretical considerations which have prompted the present investigation will be discussed fully at another time and place. Suffice it to say for the moment that rennet (as a class) appears to be widely distributed in nature, in the animal as well as in the vegetable kingdom. In the latter, its native function often seems to be that of a coagulant of the sap of the plant in which it exists, a process comparable, in some respects, to that of blood coagulation.

Our first attempt to discover the effect of pancreatic rennet upon the coagulation of the blood consisted in the following simple experiment. A small quantity of the purified pancreatic rennet was added to a portion of blood freshly drawn from the cubital vein of an hemophilic individual, and the coagulation time noted. We found that whereas the blood in the control tube required 1 hour and 20 minutes for coagulation, the specimen to which the rennet was added clotted in 90 seconds. The result was so striking that we determined to make a careful investigation of the phenomenon.

In a study of the effect of various tissue extracts on blood coagulation, Mills[2] found that a saline extract of pancreas has very slight coagulating power for blood. The coagulating substance which Mills isolated from various organs other than the pancreas he identified as a protein-lipin. It might be added that thus far we have not been able to demonstrate any lipin element in our pancreatic rennet preparations. Therefore, it appears that our rennet is not identical with the substance which Mills isolated.

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[1] Epstein, A. A., PROC. SOC. EXPER. BIOL. AND MED., 1921, xix, 3.

[2] Mills, C. A., *J. Biol. Chem.*, 1919, xl, 425.

To determine the efficiency of pancreatic rennet as a blood coagulant experiments were performed both in vitro and in vivo. The test tube method of Lee and White[3] was used for estimating the coagulation time. In the animal experiments, the individual specimens were withdrawn from different vessels.

We will first consider the effect of refined pancreatic rennet on normal human blood. For this, blood was obtained from the cubital vein of a normal individual in a series of small test tubes, some containing $CaCl_2$, others $CaCl_2$ and pancreatic rennet, and still others containing rennet alone. The two controls which were made, one at the beginning and one at the end of the experiment, showed the coagulation time to be 11 and 12 minutes respectively. The addition of $CaCl_2$ to the blood reduced the coagulation time to a period ranging between 5 and 8 minutes. Pancreatic rennet alone reduced the coagulation time to 1 and 1½ minutes respectively. Calcium chloride and pancreatic rennet, together, short-

TABLE I.

EXPERIMENT 1.

*a. Effect of $CaCl_2$ 1 per cent. on the Coagulation of Whole Blood.*

| | | | |
|---|---|---|---|
| Whole blood | 1 c.c. | 1 c.c. | 1 c.c. |
| $CaCl_2$—1 per cent | 1 gtt. | 1 gtt. | 2 gtt. |
| Norm. saline | 1 gtt. | 2 gtt. | 3 gtt. |
| Clotting time | 8 min. | 7 min. | 5 min. |

*b. Effect of Pancreatic Rennet on the Coagulation of Whole Blood.*

| | | | | |
|---|---|---|---|---|
| Whole blood | 1 c.c. | 1 c.c. | 1 c.c. | 1 c.c. |
| Pan. rennet | 1 gtt. | 2 gtt. | 3 gtt. | 4 gtt. |
| Saline | 1 gtt. | 1 gtt. | 1 gtt. | 1 gtt. |
| Clotting time | 1 min. | 1 min. | 1 min. | 1½ min. |

*c. Effect of $CaCl_2$ and Pancreatic Rennet on the Coagulation of Whole Blood.*

| | | | | |
|---|---|---|---|---|
| Whole blood | 1 c.c. | 1 c.c. | 1 c.c. | 1 c.c. |
| $CaCl_2$—1 per cent | 1 gtt. | 1 gtt. | 1 gtt. | 1 gtt. |
| Pan. rennet | 1 gtt. | 2 gtt. | 3 gtt. | 4 gtt. |
| Clotting time | 30 sec. | 15 sec. | 15 sec. | 30 sec. |

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[3] Lee, R. I., and White, P. D., *A. J. Med. Sci.*, 1913, cxlv, 495.

ened the coagulation time of this blood to 15 and 30 seconds respectively. (Expt. 1, Table I.)

We then tested once more the effect of the pancreatic rennet on the blood of a hemophiliac and the results show (Expt. 2, Table II) that the coagulation time could be reduced from 1 hour and 15 minutes to 2 minutes, by very small, but adequate amounts of the rennet.

TABLE II.

EXPERIMENT 2.

*Effect of Pancreatic Rennet on the Coagulation Time of Hemophiliac Blood.*

The control tests showed the clotting time of hemophiliac blood to be 1 hour and 15 minutes at the beginning of the experiment and 1 hour and 20 minutes at the end.

| | | | | |
|---|---|---|---|---|
| Hemophiliac blood | 1.0 c.c. | 1.0 c.c. | 1.0 c.c. | 1.0 c.c. |
| Activated pan. extract—1 per cent | 0.3 c.c. | 0.2 c.c. | 0.15 c.c. | 0.1 c.c. |
| (Rennet content) | 0.003 c.c. | 0.002 c.c. | 0.0015 c.c. | 0.001 c.c |
| Clotting time | 2 min. | 4 min. | 20 min. | 29 min. |

We next tested the effect of pancreatic rennet on citrated normal plasma. In this experiment, as in that with whole blood, we find that the rennet accelerates coagulation. The clotting time is reduced from approximately 8 minutes to 30 seconds. Two other facts come to light in this experiment, first, that the shortening of the coagulation time is proportionate to the concentration of the rennet, and secondly, that the rennet is capable of coagulating citrated plasma without the addition of calcium chloride (see Table III, Exp. 3, Column 3). The exact manner in which the rennet accomplishes this result cannot be stated with certainty. In the normal coagulation of blood, calcium is indispensable. It will be recalled that rennet requires calcium for the coagulation of milk. Hence, it must be assumed that rennet causes the clotting of citrated plasma, either in a manner peculiar to itself, or that it accomplishes it by causing a dissociation of the calcium ion from the citrate molecule. The experiment indicates, however, that the addition of extra calcium, in the form of chloride, enhances the coagulating effect of the pancreatic rennet on citrated plasma.

The intravenous injection of large doses of activated crude pancreatic extract, or the purified rennet in rabbits is not accompanied by anaphylaxis or other symptoms. Intravascular clotting does not occur as is the case with saline lung extracts or other tissue coagulants.

### TABLE III.

#### EXPERIMENT 3.

| | | | | | | |
|---|---|---|---|---|---|---|
| Diluted citr. plasma | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. |
| $CaCl_2$—1 per cent | 2 gtt. | 2 gtt. | 0 | 2 gtt. | 2 gtt. | 2 gtt. |
| Pan. rennet—1 per cent | 0 | 0 | 0.3 c.c. | 0.1 c.c. | 0.2 c.c. | 0.3 c.c. |
| Clotting time | 8 min. | 7 min. | 8 min. | ¾ min. | ½ min. | ½ min |

#### EXPERIMENT 4.

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Citrated plasma | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. |
| $CaCl_2$—1 per ct. | 2 gtt. | 2 gtt. | 2 gtt. | 2 gtt. | 2 gtt. | 2 gtt. | 2 gtt. | 2 gtt. | 2 gtt. | 2 gtt. |
| Rennet | 0 | 0.125 | 0.062 | 0.031 | 0.015 | 0.075 | 0.038 | 0.014 | 0.007 | 0.003 |
| Saline | 0.25 | — | — | — | — | — | — | — | — | — |
| Clotting time | 6½ m. | 2 m. | 2 m. | 1½ m. | 2 m. | 3½ m. | 4½ m. | 5 m. | 5½ m. | 6 m. |

Cats were used in studying the effect of intravenous injection of pancreatic rennet on blood coagulation. Moderate doses of the rennet reduce the coagulation time, but not as strikingly as in the experiments conducted in vitro. Excessive doses prolong the coagulation, without giving rise to intravascular clotting. In this respect also, the pancreatic rennet differs from the tissue coagulants studied by Mills. The underlying mechanism of the shortening and prolongation of the clotting time thus produced is being further investigated.

The specific effects produced by the intravenous injection of activated crude pancreatic extract, and the refined rennet, is illustrated by the following experiments.

#### EXPERIMENT 5.

18 c.c. of a 3 per cent. activated crude pancreatic extract solution (rennet content 0.0054 g.) were injected into the jugular vein of a cat (under ether anesthesia). The results obtained are shown in the following protocol.

| | |
|---|---|
| Clotting time before injection | 7 minutes. |
| Clotting time 5 minutes after injection | 4 minutes. |
| Clotting time 10 minutes after injection | 2 minutes. |
| Clotting time 20 minutes after injection | 3 minutes. |
| Clotting time 25 minutes after injection | 3 minutes. |
| Clotting time 40 minutes after injection | 3 minutes. |

### EXPERIMENT 6.

*Effect of a Large Dose of Refined Pancreatic Rennet on the Coagulation of the Blood (Cat).*

4 c.c. of a refined pancreatic rennet (equal to 38 c.c. of a 3 per cent. sol. of the crude sol.) was used.

| | |
|---|---|
| Clotting time before injection | 5½ minutes. |
| Clotting time 2 minutes after injection | 3 minutes. |
| Clotting time 5 minutes after injection | 5 minutes. |
| Clotting time 10 minutes after injection | 15 minutes. |
| Clotting time 25 minutes after injection | 6 minutes. |

Thus we find after an initial diminution of the clotting time there was a prolongation and a return to normal at the end of 25 minutes.

## THE EFFECT OF RENNETS FROM OTHER SOURCES ON THE COAGULATION OF BLOOD.

On account of the striking action of pancreatic rennet in hastening of coagulation of the blood in vitro, studies have been undertaken with rennets from other sources, for purposes of comparison. The action of these rennets on blood coagulation may possibly be less potent than that of the pancreatic variety. The isolation of the pure rennet from the stomach has thus far been difficult to accomplish. In one experiment we used a crude pepsin solution of known milk coagulating power and found a distinct delay in the coagulation of the blood (see Exp. 7). This action may possibly be due to the impurities in the crude pepsin-rennet solution.

### EXPERIMENT 7.

| | | | | | |
|---|---|---|---|---|---|
| Citrated plasma | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. | 0.5 c.c. |
| Crude pepsin—5 per cent | 0 | 1 gtt. | 2 gtt. | 3 gtt. | 4 gtt. |
| $CaCl_2$—1 per cent | 2 gtt. | 2 gtt. | 2 gtt. | 2 gtt. | 2 gtt. |
| Clotting time | 8 min. | 7 min. | 25 min. | none | none |

at the end of 24 hours.

## CONCLUSIONS.

1. Pancreatic rennet shows great activity as a coagulant for normal and hemophilic blood.

2. Intravenous injection of pancreatic rennet does not produce anaphylaxis.

3. Moderate intravenous doses diminish the coagulation time, and excessive doses increase the coagulation time after an initial shortening.

4. Intravascular clotting has not been observed after intravenous administration of pancreatic rennet.

### 43 (1790)

## Observations on the excretion of sugar in the urine in health and disease.

By **LUDWIG KAST** and **HILDA M. CROLL.**

[*From the Department of Medicine and the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York City.*]

The amount of sugar in the twenty-four-hour urine specimens has been determined by the new acetone-picric acid method of Benedict and Osterberg.[1] It was found that a diet rich in carbohydrate increases the amount of sugar excreted over that on a low carbohydrate diet. This method also demonstrated the increase in hourly sugar excretion after meals and after glucose ingestion. In four normal adults studied, the average amount of reducing sugar excreted daily was between 0.59 and 1.14 grams. In ten children from 2.5 to 9 years of age, representing a variety of pathological conditions, the average range was from 0.12 to 0.43 gram sugar daily. To cite a few of the 116 hospital cases studied, in 20 patients diagnosed as neurasthenics, on the average between 0.42 and 1.24 grams sugar were excreted daily; in hyperthyroidism the average range was between 0.46 and 0.98 grams; in one case of hypothyroidism an average of 0.40 gram sugar was excreted; in nephritis 0.41 to 0.89 grams, in hypertension 0.44 to 1.12 grams, in arthritis 0.44 to 1.39 grams, and in various cardiac disturbances 0.51 to 1.39 grams on the average were excreted daily. It appears, therefore, that in the diseases studied, when the patients are on ordinary diets, there is no striking increase nor decrease over normal urinary excretion. In diabetes alone there is an increase, although when by dietary regulation the patient is rendered "sugar free" the amount of sugar excreted is practically normal.

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[1] Benedict, S. R., and Osterberg, E., *J. Biol. Chem.*, 1921, xlviii, 51.

### 44 (1791)

## Isopropyl alcohol, a convenient laboratory anesthetic for cats.

By DAVID I. MACHT.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

In connection with a comparative study of normal and secondary alcohols the author had occasion to inquire into their comparative narcotic properties. It was noted that when a suitable dose of isopropyl alcohol, a drug which is comparatively cheap, is administered to cats by the stomach tube, a general anesthesia is produced lasting for many hours and indeed in some cases for several days. In order to use this drug as an anesthetic for cats, the animals must first be completely anesthetized with ether, a stomach tube is then passed and a dose of isopropyl alcohol from 5 to 5½ c.c. per kilo weight of the animal is introduced into the stomach together with two or three times its volume of water. The stomach must be empty before the administration of the drug. The brief-lasting stage of ether isopropylol anesthesia is quickly followed by a complete narcosis, produced and maintained by the isopropyl alcohol alone. Indeed it is usually not necessary to take the cats off the table after the administration of the drug by stomach tube. The blood pressure curve obtained with such animals is remarkably high and the circulation is certainly much less depressed than by certain chlorinated hypnotics which have been used as anesthetics for cats. The effect on the respiration in larger doses than above is more depressant but when the proper dose is administered the animals continue to live with very good circulation and satisfactory respiration for many hours.

### 45 (1792)

## Apparatus for micro-manipulation and micro-injection.

By ROBERT CHAMBERS.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

This apparatus is designed for the purpose of dissecting living cells or injecting substances into them, and for isolating microorganisms. Its advantage over that which Barber described in the *Philippine Journal of Science* in 1914 is its simplicity of construction, and the accuracy with which it can be manipulated.

The apparatus consists of two instruments, the micro-manipulator for producing movements in the microscopic field in any of three dimensions and, second, the micro-injection instrument for securing the necessary pressure to drive or suck substances through a micro-pipette. The method of making glass micro needles and pipettes is given in full in Barber's paper and in mine in the *Biological Bulletin* of 1918.

The micro-manipulator is small and compact and can be attached to the stage of any microscope. It consists of a system of rigid metal bars connected together with spring hinges. By turning certain screws the bars are forced apart. On reversing the screws the springs return the bars to their original positions. The instrument moves the tip of a needle or a pipette in three arcs at right angles to one another. The arcs are small enough so that, in the microscopic field, the needle moves practically in straight lines. The movements are fine and steady enough to be under perfect control when viewed under the highest power of the microscope. The instrument can be used singly for one needle only or with a companion when two needles, or a pipette and a needle, are to be used simultaneously.

In the micro-injection instrument mercury or an inert oil (Nujol) is used to procure the necessary pressure. The instrument consists of a thin-walled steel tube about six inches long and half an inch in diameter, one end of which is provided with a stopcock. The other end leads into a small steel tube fine enough to be flexible and long enough and so bent that, while the large tube lies on the table beside the microscope, the tip of the fine tube can be held in the pipette carrier of the micro-manipulator. Into this tip a glass Barber pipette is sealed. Mercury or oil is introduced through the stopcock of the large tube and is forced on into the micro-pipette. The stopcock is then shut off. By means of leverage clamps on the thin-walled tube the mercury or oil can be driven through a pipette having an aperture of only one micron in diameter. By turning the screws of the micro-manipulator the tip of the pipette can be brought into a hanging drop in a

Barber's moist chamber. Release of pressure on the steel tube draws substances into the pipette. Injection and suction in microscopic quantities is accurately controllable as the meniscus of the mercury or oil in the pipette responds instantly to the pressure of the leverage clamps.

### 46 (1793)

## The effect of experimentally induced changes in consistency on protoplasmic movement.

By ROBERT CHAMBERS.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

Agitation by means of a micro-dissection needle tends to cause the protoplasm of a living cell to pass from a more solid to a less solid phase.

In marine ova, where one can closely follow the solidifying of the protoplasm just prior to cell division, mechanical agitation will cause the protoplasm to revert to its original liquid state so that the egg reverts to the shape of a sphere. If the egg so treated be subsequently left undisturbed the solidifying process starts up again with the result that the egg undergoes normal cleavage.

In a previous communication[1] the writer has described the structural relations of changes in protoplasmic consistency of the *Amœba* to the formation of pseudopodia. The maintenance of pseudopodia depends upon a relatively solid state of certain parts of the *Amœba*.

A resting *Amœba*, with numerous slender pseudopodia all over its surface, is relatively solid. Upon mechanical agitation the pseudopodia are retracted as the *Amœba* becomes more liquid. Fresh pseudopodia in an agitated *Amœba* tend to be broad lobate and, if the agitation be continued, all of the *Amœba* liquefies. The entire body then becomes, as it were, a single pseudopodium with a peripheral current of granules flowing away from its anterior end and a central current flowing forward. An *Amœba* in this extreme state does not change in position as the back flow tends to equal the forward flow. *Amœbæ* which are experimentally brought into this state have, so far, not been observed to return to their previous condition. The rate of flow of the currents gradually slows down until the animal dies.

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[1] Chambers, Robert, PROC. SOC. EXP. BIOL. AND MED., 1920, xviii, 66.

The protoplasm of an *Amœba* exists in a certain normal state of consistency from which it may deviate so as to solidify on the one hand or liquefy on the other. This normal state may be shifted not only by agitating the *Amœba* but also by injecting certain solutions. This I have been able to do with hydrochloric acid and with sodium hydrate.

A trace of acid throws the normal state to the more solid side, while the alkali throws it to the more liquid side. An acidified *Amœba* forms long slender pseudopodia because the peripheral back flow in the developing pseudopodium is quickly arrested by a setting of the protoplasm. The area of the base of the pseudopodium is, therefore, quickly limited and the extending pseudopodium conforms to this narrow base. In an alkalinized *Amœba*, on the other hand, the peripheral back flow of a developing pseudopodium tends to be arrested much more slowly. As a result of this the base of the pseudopodium spreads considerably before the protoplasm sets. The extending pseudopodium, having a larger base upon which to build, then becomes broadly lobate.

These observations harmonize with my experiments on injecting "acid" and "basic" organic dyes. The basic dyes, which contain a relatively strong acid radicle, jelly the protoplasm, whereas acid dyes, with a strong basic radicle, liquefy it.

It is interesting to note that these changes can be brought about in protoplasm while it is yet alive and that one can thereby change the character of the pseudopodia produced.

### 47 (1794)

## Alterations in the cardiac mechanism after administration of quinidine to patients with auricular fibrillation.[1]

By **ROBERT L. LEVY.**

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

Sufficient evidence is now at hand to indicate that in a certain number of patients suffering from fibrillation of the auricles

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[1] This paper was presented at the One Hundred Thirteenth meeting of the Society for Experimental Biology and Medicine, October, 1921.

(about 50 per cent.), oral administration of quinidine sulphate serves to restore the normal rhythm. It is the purpose of this communication to record the mechanism of the heart's action which has been observed in the first eleven patients who have received quinidine in this hospital.

Five hundred and seven electrocardiograms taken on eleven patients have been measured and analyzed. After a preliminary dose of .2 to .4 gm. of quinidine to test for the presence of an idiosyncrasy to members of the cinchona group, .4 gm. of the drug has been given by mouth, in gelatin capsules, either three times daily or every two hours, until either the establishment of normal rhythm or the appearance of untoward symptoms indicated cessation of therapy. No more than 2.0 gm. of quinidine were administered in twenty-four hours, though treatment has been continued daily for as long as ten days.

Electrocardiograms were taken in some instances as often as every five minutes during the time when a change in rhythm was anticipated. Usually curves were made at two-hour intervals on the days on which the drug was given, and at least daily throughout the periods of observation.

*Cases in which the Normal Mechanism was Restored.*—Three patients received ten courses of quinidine. Restoration of the normal mechanism was accomplished nine times. The first effect noted was usually an acceleration of ventricular rate. This was followed at times by the appearance of premature beats, arising more commonly in the right, but occasionally in the left ventricle, and at times in both. If electrocardiograms were taken at sufficiently frequent intervals, the transitional mechanisms in the common order of their appearance were: coarse fibrillation, impure flutter, flutter, and normal rhythm. This sequence was not invariably demonstrated in all its phases, and it is possible that one or more of the intermediate mechanisms may be omitted. In one patient the transition from auricular flutter to the normal rhythm was photographed in the second lead. The change was rather abrupt, there being a period of altering auricular activity, slowing of ventricular rate for several beats, a relatively long period of a systole of both auricles and ventricles and then prompt resumption of the sinus rhythm. The P waves, denoting auricular activity, were well formed, even in the first normal beats, though showing some tendency to slight alterations both in form and in voltage during the early cycles of the restored sequential mechanism.

On the day on which normal rhythm was established the rate usually fell to 80 or 90, and on the following day to 60 or 70, and subsequently remained at or about this level. During the time the normal rhythm prevailed, it was common to see occasional auricular premature beats. These, together with ventricular premature contractions, when these were present, could be abolished by giving more of the drug.

P-R (conduction) time, after appearance of the sinus rhythm, was within normal limits in two patients, and was lengthened appreciably in the third. It is not possible to say whether the drug was responsible for delaying conduction of the impulse through the auriculo-ventricular bundle in this instance.

In one patient the QRS interval, which may be taken to indicate the time of the conduction of the impulse through the ventricles, was lengthened. In two patients, after establishment of normal rhythm, there was alteration in the form of the ventricular complex, exhibiting itself commonly as a change in the voltage and contour of the R and S waves.

The T wave often tended to reverse its direction and change its voltage after the normal rhythm was restored, returning to its original direction and form after reversion to the fibrillatory mechanism.

In one patient paroxysms of ectopic ventricular tachycardia preceded the onset of impure flutter, which, in turn, was followed by the normal rhythm. In this same patient sino-auricular block and paroxysms of auricular tachycardia were also observed when the sequential rhythm prevailed.

Digitalization prior to quinidinization was not an essential factor for success in therapy, for in the same individual the normal mechanism was restored on one occasion with ventricular rate of 180 and at another time, after the administration of digitalis, when the ventricular rate ranged from 90 to 100.

The duration of the normal rhythm after a single course of quinidine varied from a few hours to 23 days. In one patient it has been possible, by means of intermittent quinidine therapy, properly spaced, to maintain the normal rhythm for over five months, with coincident marked clinical improvement.

Intravenous injection of atropine sulphate (1.0 to 1.5 mgm.) in these patients, at a time when fibrillation was present and again when the normal rhythm prevailed, resulted in the usual increase in ventricular rate, but in no significant alteration in the cardiac mechanism or in the form of the electrocardiogram.

*Cases in which Restoration of the Normal Mechanism was Not Accomplished.*—Eleven courses of quinidine were administered to 8 patients. As in the group just decsribed, tachycardia was commonly the first effect observed. Ventricular premature beats, at times in the form of coupled rhythm, were more commonly seen than in those patients in whom the sinus rhythm was eventually established. Occasionally the fibrillatory waves became coarser. In two patients auricular flutter followed administration of the drug, but a larger dosage was not followed by the normal rhythm. In one of these cases auricular flutter persisted for three days and was followed, after administration of digitalis, by reversion to the fibrillatory mechanism. Paroxysms of ectopic ventricular tachycardia occurred three times. Although of short duration, they served to indicate that quinidine as a therapeutic agent was not to be administered with impunity, for ventricular tachycardia occurring in dogs poisoned by digitalis or strophanthin is not infrequently the precursor of ventricular fibrillation.

### 48 (1795)

## Studies on the acetonuria produced by diets high in fat.

By ROGER S. HUBBARD and FLOYD R. WRIGHT.

[*From the Clifton Springs Sanitarium, N. Y.*]

The following ratio was suggested to express the ketogenic balance of any diet:

$$100 \times \frac{1.5\ (\text{weight carbohydrate} + 25\ \text{per cent. weight protein})}{95\ \text{per cent. weight fat}}.$$

This ratio is based on the relative molecular weights of glucose and the higher fatty acids—stearic, palmitic, and oleic; in it it is assumed that approximately half the glucose derived from protein is used up in burning the ketogenic material from the $\alpha$-amino acids leucine, tyrosine and phenyl alanin occurring in the same protein; no allowance is made in the expression for the possible antiketogenic effect of the glycerol radicle present in the fats.

Diets high in fat were fed to a normal subject, and to arthritic patients undergoing the Pemberton[1] treatment. These diets were based on that suggested by Shaffer[2] which contained 10 per cent. of the total calories as protein, 10 per cent. as carbohydrate, and 80 per cent. as fat. The degree of acetonuria which corresponded with each diet was determined, and the results compared with the numerical values of this ratio.

From a study of these values which corresponded with a very mild degree of acetonuria it was concluded: one, that the phenomenon of ketogenesis could properly be regarded as a molecular reaction between ketogenic and antiketogenic compounds in the diet; two, that protein entered into the reaction only to the extent of the glucose which could be drived from the $\alpha$-amino acids contained in it; three, that the glycerol radicle of fat figured as a source of antiketogenic material only to the extent to which glucose could be derived from it; and, probably, four, that the glycerol radicle probably did figure as a source of antiketogenic material to the extent to which it could yield glucose.

### 49 (1796)

## The glycogen content of the tissues of diabetic animals and the influence of adrenalin thereon.

By **A. I. RINGER, H. DUBIN** and **F. HULTON FRANKEL.**

[*From the Department of Physiological Chemistry, University of Pennsylvania, Philadelphia, Penn.*]

In a series of experiments on dogs rendered diabetic by means of phlorhizin, the glycogen content of the muscles was studied immediately after the animals were killed. The muscles of thirteen animals were analyzed at the end of two days of glucosuria.

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[1] Pemberton, R., *Am. J. Med. Sci.*, 1917, cliii, 678.

[2] Shaffer, P. A., *J. Biol. Chem.*, 1921, xlvii, 449; Woodyatt, R. T., *Arch. Int. Med.*, 1921, xviii, 125.

The average glycogen content was 482 mgs. per one hundred grams of muscle. The muscles of eleven animals at the end of the third day of glucosuria contained an average of 306 mgs. per one hundred grams. At the end of the fourth day, the muscles of twelve animals contained an average of 228 mgs. of glycogen per one hundred grams. At the end of the fifth day seven animals showed an average of 155 mgs. per one hundred grams of muscle, and at the end of the seventh day two animals showed the presence of 124 and 151 mgs. per one hundred grams of muscle.

The detailed results are summarized in Table I.

TABLE I.

GLYCOGEN CONTENT OF MUSCLE OF DIABETIC DOGS.

Days of Glucosuria.

| | 2 | 3 | 4 | 5 | 7 |
|---|---|---|---|---|---|
| | 723 | 250 | 181 | 111 | 124 |
| | 626 | 381 | 237 | 097 | 151 |
| | 573 | 337 | 226 | 080 | |
| | 415 | 499 | 200 | 315 | |
| | 430 | 236 | 144 | 293 | |
| | 325 | 305 | 130 | 110 | |
| | 295 | 121 | 120 | 105 | |
| | 447 | 192 | 297 | | |
| | 435 | 356 | 282 | | |
| | 528 | 378 | 319 | | |
| | 628 | 306 | 316 | | |
| | 407 | | 286 | | |
| | 440 | | | | |
| Average.... | *482* | *306* | *228* | *155* | *138* |

Throughout these periods, the animals fasted and were given phlorhizin injections by the Coolen method (one gram daily in olive oil).

These findings prove that in spite of *continuous fasting and in spite of complete diabetes, the muscle cells will hold on to a certain amount of glycogen, which we may term residual glycogen.*

In a second series of experiments, diabetic phlorhizinized dogs were given injections of three to seven milligrams of adrenalin, and were killed twenty-four hours after that. The injections were made on the second or third day of glucosuria.

The analysis of the muscles of five dogs of this series showed that absolutely no glycogen was present in them, proving that *adrenalin will cause a complete discharge of all the glycogen from the muscle cells.*

In a third series of experiments, diabetic dogs were rendered glycogen free as in the second series. They were then given substances like glycocoll and alanin, propionic and lactic acids, all of which are known to be converted quantitatively into glucose. Not in one single instance was the glucose elimination from the adrenalinized animals in any way comparable to the amounts that are excreted by animals not treated with adrenalin. In other words, either there is interference with sugar formation in the absence of the "residual" glycogen or the glucose that is formed from glucogenetic substances is utilized by these cells, and not excreted in the urine.

Table II is illustrative of this point.

TABLE II.

INFLUENCE OF DEGLYCOGENIZATION ON EXTRA GLUCOSE FORMATION FROM LACTIC AND PROPIONIC ACIDS.

*Experiment 92. Phlorhizinized Dog. Twelve Hour Periods.*

| Period. | Weight in Kg. | N. | G. | G : N. | Extra Glucose. | Remarks. |
|---|---|---|---|---|---|---|
| I........ | 13.68 | 2.34 | 20.54 | 8.78 | | First day of phlorhizin glucosuria. |
| II....... | | 4.29 | 42.52 | 9.92 | | 6 mg. adrenalin injected. |
| III...... | 13.23 | 6.77 | 21.86 | 3.08 | | 6 mg. adrenalin injected. |
| IV....... | | 6.66 | 18.66 | 2.80 | | |
| V....... | 12.83 | 7.29 | 20.59 | 2.83 | | |
| VI....... | | *6.04* | *22.40* | *3.71* | *4.28* | 9.0 gms. of lactic acid as sodium salt dissolved in 24 c.c. water given subcutaneously. |
| VII...... | 12.20 | 5.62 | 17.97 | 3.20 | | |
| VIII..... | | 6.47 | 20.70 | 3.20 | | |
| IX...... | 11.95 | 5.79 | 17.58 | 3.04 | | |
| X....... | | 4.88 | 14.72 | 3.02 | | |
| XI...... | 11.48 | *4.80* | *18.38* | *3.83* | *2.75* | 7.4 gms. propionic acid as sodium salt dissolved in 20 c.c. water given subcutaneously. |
| XII..... | | 4.41 | 15.40 | 3.50 | | |
| XIII..... | | 4.51 | 15.16 | 3.36 | | |

At end of period XIII, ether anesthesia, animal bled to death. Glycogen in muscles 0.039, 0.038 per cent.

In this experiment the giving of adrenalin during period II is followed by a sweeping out of all the residual glycogen, as is shown by the high G : N ratio. The giving of adrenalin during period III is followed by no more extra glucose elimination. In period VI, 9.0 grams (M/10) of lactic acid as sodium salt was administered subcutaneously. Only 4.28 grams of extra glucose were excreted in the urine, which is exactly one half of that which is usually obtained. In period XI 7.4 grams (M/10) of propionic acid were given subcutaneously. During this period only 2.75 grams of extra glucose were excreted, which is less than one third of the usual.

In 1913, Ringer and Frankel[1] found that the administration of acetaldehyde and propyldehyde to phlorhizinized dogs was followed by a marked drop in the formation of acetone bodies and by a rise in the glucose elimination. Explanation for the formation of sugar after propylaldehyde could be found very easily by assuming direct conversion, since it had already been established that both propylalcohol and propionic acid could give rise to glucose, but for glucose formation directly from acetaldehyde no chemical basis could be found. They therefore had to look for other channels of glucose formation after acetaldehyde administration. They suggested the possibility that the marked antiketogenetic effect of acetaldehyde and glucogenetic effect might be coupled together and that it was possible for acetaldehyde in the body to form a chemical union with either $\beta$-hydroxybutyric acid or acetoacetic acid, forming a compound which is glucogenetic. This hypothesis seemed to harmonize with all the facts and seemed to explain both the antiketogenetic effects of the acetaldehyde as well as the glucogenetic.

About a year later Sansum and Woodyatt[2] published a series of experiments in which they showed that the administering of ether or nitrous oxide by inhalation to phlorhizinized animals was followed by a marked increase in the glucose excretion, proving that the animals were not entirely glycogen free. They then treated their animals with adrenalin, to the point when they no longer excreted extra glucose, and then gave them acetaldehyde. The administration of acetaldehyde to those dogs was followed by very insignificant or no extra glucose elimination. From these experiments they concluded that acetaldehyde acts similarly to ether and nitrous oxide, by virtue of its hypnotic effects.

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[1] Ringer, A. I., and Frankel, E. M., *Jour. Biol. Chem.*, 1914, xvi, 563–579.

[2] Sansum, W. D., and Woodyatt, R. T., *Jour. Biol. Chem.*, 1915, xxi, 1–21.

We cannot accept Sansum and Woodyatt's application of their results to acetaldehyde for a number of reasons.

I. Because in the doses given, acetaldehyde has no hypnotic effects, whereas the ether and nitrous oxide render the animals unconscious.

II. The giving of ethyl alcohol and a number of other substances to the point of complete hypnosis did not produce any extra glucose elimination, proving that it is more than hypnosis that is affecting the residual glycogen.

III. In the third series of our experiments we found that substances like glycocoll, alanin, lactic and propionic acids when administered to adrenalinized dogs yield less than one half the amount of glucose than they do ordinarily, and that a number of glucogenetic substances would never have been detected if Sansum and Woodyatt's technique were followed.

Glycogen is the most mobile food stuff that the body possesses, and if we find that after five and seven days of starvation and complete diabetes the body cells still cling to this residual glycogen, in spite of that tremendous demand for it, and still hold on to about 150 mgs. per 100 grams of muscle, it must have a different significance in the cell economy from the ordinary glycogen that moves in and out of the cell. After an animal is deglycogenized by means of adrenalin there must be established a state of ''glycogen hunger.'' When a glucogenetic substance is given during that period we can readily conceive of that glucose being retained in part to supply that glycogen. This is how we would interpret the failure of Sansum and Woodyatt to obtain extra glucose from acetaldehyde.

In a fourth series of experiments we rendered dogs glycogen free as described by Sansum and Woodyatt and which method we have proven in the second series of these experiments actually does free the animals from glycogen. We then allowed the animals to continue fasting and be diabetic by means of phlorhizin. The animals were killed three days after the deglycogenization and the muscles were found to contain the following amounts of glycogen, 0.020, 0.033, 0.023 and 0.039 gm. per 100 grams. In one animal which was kept for five days after deglycogenization 0.069 gram of glycogen was found per 100 grams of muscle.

These experiments prove that fasting diabetic dogs, even after they have been completely deglycogenized, possess the power of glycogen formation. Glucogenetic substances therefore can well be administered to animals without giving rise to extra glucose in the urine.

### CONCLUSION.

I. Diabetic dogs contain glycogen in their muscles to the extent of 0.150 per cent. even after seven days of fasting and diabetes; residual glycogen.

II. This glycogen can be completely driven out by means of adrenalin.

III. Deglycogenized diabetic animals even during a period of prolonged fasting and diabetes are capable of reforming their lost residual glycogen.

IV. Failure on the part of an animal to show extra glucose elimination during the period of deglycogenization does not mean that the substance is not glucogenetic.

V. The conclusions of Sansum and Woodyatt that acetaldehyde is not glucogenetic nor antiketogenetic are objected to as invalid.

## 50 (1797)

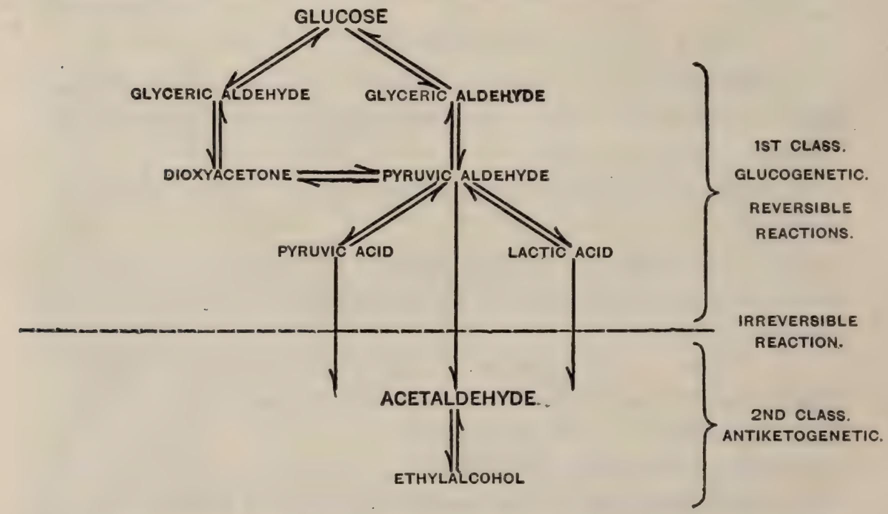
## Concerning antiketogenesis.

By **A. I. RINGER.**

[*From the Montefiore Hospital, New York City.*]

It is a well-known fact that the withdrawal of carbohydrates from the diet of normal individuals is followed by the appearance of ketone bodies in the urine. Individuals that have interference with their power to utilize carbohydrates, as diabetics, develop degrees of ketonuria that are proportional to the severity of the disturbances in their carbohydrate metabolism. It is also established definitely that these ketone bodies are formed normally in the intermediary metabolism of fat and of certain amino acids, and that with the oxidation of carbohydrates the ketone bodies suffer oxidation. The carbohydrates therefore are known as antiketogenetic.

For a number of years we have been engaged in trying to solve the problem how the carbohydrates exercise their antiketogenetic powers, and to find a chemical explanation for it. We fed to diabetic animals every known chemical compound that may play a rôle in the intermediary metabolism of carbohydrates, and we found that they may be divided into two classes.


GLUCOSE
GLYCERIC ALDEHYDE
GLYCERIC ALDEHYDE
DIOXYACETONE
PYRUVIC ALDEHYDE
PYRUVIC ACID
LACTIC ACID
ACETALDEHYDE
ETHYLALCOHOL
1ST CLASS.
GLUCOGENETIC.
REVERSIBLE
REACTIONS.
IRREVERSIBLE
REACTION.
2ND CLASS.
ANTIKETOGENETIC.

The first consisting of those substances like glyceric aldehyde, dioxyacetone, pyruvic aldehyde, pyruvic acid and lactic acid, which when given to diabetic animals are completely and directly converted into glucose. They all possess the power of reversible reaction in the body, *i.e.*, they all can be converted from one into the other, and possess but slight antiketogenetic properties because when given to diabetics the main force of the reaction is upwards towards the glucose stage, and as such they become excreted in the urine. Practically none of these are burnt in the body of the diabetic animal.

The second consisting of substances like acetaldehyde and perhaps also ethylalcohol. The reaction towards these from glucose and its intermediary products is irreversible, when given to diabetics, they possess marked antiketogenetic powers. For acetaldehyde this was established by Ringer and Frankel and for alcohol by Neubauer and by Benedict and Török.

In diabetes the intermediary metabolites of glucose seem to have lost the power to break through the trapdoor which leads to the acetaldehyde stage in which they are capable of exercising antiketogenetic effects. Apparently the only way the carbohydrates affect ketogenesis is when they have come down to the acetaldehyde stage.

The modus operandi of the action of acetaldehyde on the ketone bodies has already been discussed by Ringer and Frankel. They suggested the possibility of acetaldehyde combining with $\beta$-hydroxybutyric acid or acetoacetic acid giving rise to a substance which is not ketogenetic.

### 51 (1798)

## Botulism.[1] A method for determining the thermal death time of the spores of Bacillus botulinus.

By ERNEST C. DICKSON and GEORGINA S. BURKE.

[*From the Laboratory of Experimental Medicine, Stanford University Medical School, San Francisco, Calif.*]

In the course of a series of experiments dealing with the determination of the thermal death point of spores of *Bacillus botulinus* in which the method of procedure recommended by Bigelow and Estey[2] was followed with minor modifications, it was found that in the daily transplanting of several hundred specimens to the tubes in which the heated material was to be incubated, it was inevitable that a certain small percentage of the tubes became contaminated. The number of proved contaminations was not large, less than 1 per cent. in a test of approximately 2,000 tubes, but because of the fact that one could not be absolutely certain that any particular tube was free from contamination, it was impossible to draw accurate conclusions in any instance where an unusual survival time was indicated. It was therefore imperative that a method be devised in which the necessary number of tubes per day could be handled with rapidity, and, at the same time, with absolute protection against any possibility of contamination after their contents had been subjected to the heating process.

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[1] These experiments are a part of an investigation of Botulism which is being made in California by the U. S. Public Health Service, Leland Stanford Junior University and the University of California under a grant from the National Canners' Association, the Canners' League of California and the California Olive Association.

[2] Bigelow and Estey, *Jour. Infect. Dis.*, 1920, xxvii, 602.

After a number of trials we have adopted the following method of procedure in our experiments, and, although it cannot be adapted to the investigation of all the problems which present themselves in thermal death time experiments, it has proved to be satisfactory for the problems under immediate investigation.

Soft glass tubes, 10 x 150 mm., are used in the experiments. Three cubic centimeters of 1 per cent. glucose peptic digest liver broth, adjusted so that the final $P_H$ is between 7.3 and 7.5, are placed in each tube and covered with a thin layer of oil to prevent evaporation. The medium is sterilized at fifteen pounds pressure for thirty minutes.

Immediately before they are to be inoculated the tubes of broth are exposed to live steam for twenty minutes to expel the air. A known number of spores is added to each tube in ½ c.c. of the medium in which they have grown, the number of spores in the suspension being determined by actual count in a counting chamber. The tubes are then sealed in an oxygen flame and are ready for heating.

Although it has been found by actual counts that there is no appreciable change in the number of spores within five hours after they have been placed in the tubes, not more than ninety minutes are allowed to elapse after the tubes are inoculated before they are submitted to the heat.

The spores are heated by immersing the sealed tubes in racks into oil which is maintained constant at the required temperature and vigorously agitated. At the end of the required time of heating the tubes are removed from the oil, placed in deep pans of cold water to cool and immediately labelled. They are then incubated at 37.5° C.

The appearance of growth in the sealed tubes is characteristic and easily detected. Incubation is continued for at least ten days after growth is recognized in each instance to allow time for the formation of toxin, after which the tubes are opened under sterile precautions, deep agar, broth and meat mediums are inoculated for the purpose of observing the cultural characteristics and guinea pigs are immediately injected for the demonstration of toxin and determination of its type. No test is considered positive unless the broth culture within the sealed tube contains a virulent *botulinus* toxin at the time the tube is broken.

This technic, because it eliminates all possibility of contamination of the contents of the tubes after they have been subjected to the required amount of heat, ensures that any bacteria which are alive within the tube must be the growth from bacteria or spores which have survived the heating process. It also demonstrates beyond any possibility of doubt not only when the *botulinus* spores have survived the given exposure to heat but whether they have retained their ability to form toxin.

# SCIENTIFIC PROCEEDINGS.

## Abstracts of Communications.

### One hundred nineteenth meeting.

*Rockefeller Institute for Medical Research, December 21, 1921.*

*President Wallace in the chair.*

52 (1799)

### The distentive agencies in the growth of the cell.

By D. T. MACDOUGAL.

[*From the Desert Laboratory, Tucson, Arizona.*]

The newly separated vegetative cell including that of the simpler animals such as the Protozoans is a minute mass of protoplasm in which there are at first no lacunæ or breaks of any size, and the externally bounding layer is of extreme thinness and tenuity. The enlargement of expansion of the protoplast in this stage is chiefly one of formation or accretion of additional colloidal material and its hydration to a point where the water content is 50 to 500 times that of the dry weight of the included material.

My experiments of the last five years show that substances known to accelerate or facilitate growth also carry the hydration of living and dead cell masses and of pentosan-protein colloidal masses to a point beyond that which may occur in pure water. Such amino compounds, acidic and basic, as asparagin, alanin, glycocoll, phenyl-alanin and histidin, hydroxides and chlorides of potassium, sodium, magnesium and calcium in concentrations from 0.001 M. to 0.000,01 M. hydrochloric acid in the same range and water-soluble B yeast-vitamine Harris are included in the list and these are concentrations of biological occurrence. The enlargement of the cell in this stage must be due directly to imbibition and swelling as the vacuoles are not yet formed, although the fundamental unity of swelling and osmosis is to be recognized. In a second phase of growth, characteristic of plants and well illustrated by yeasts and bacteria, syneretic cavities appear in the plasmatic mass, and these known to the morphologist as vacuoles enlarge presumably by osmotic action, to such extent that their volume may comprise three fourths of the space of the cell.

Substances of four main groups, carbohydrates, proteins, soaps and lipoids which may be in the colloidal state of a reversible gel are entangled or intermixed in the protoplasmic mass. The components which tend to lower surface tension most would be carried to the surface, and it is to such implied causes that we must look in determining the character and derivation of the so-called plasmatic membrane, the permeability of which is of the greatest importance in the physical action of the cell, and of the wall which encloses the whole. Much controversy has raged as to nature and composition of this membrane. Its composition and character must inevitably depend upon the character of the colloidal mass which it bounds and from which it was formed. Morphologically inseparable, it yields no molecular features under the ultramicroscope, which would distinguish it from the mass. That it is a polarizable protein layer, or pentosan anhydride or that it is an albumen-lipoid mosaic are all theses which fall to the ground when tested by the known laws of colloidal action, or by the phenomena of cell-behavior.

Furthermore the conclusions of Hansteen-Cranner[1] that the ground substance of living matter is a colloidal meshwork of lipoidal matter which runs through walls from cell to cell and which takes the form of a highly irregular deposit between wall and plasma, may be taken as a highly particularized interpretation of facts he has uncovered as to the occurrence of these lipoids. All jellies present will be entangled in the protoplasmic mass, and what may be or what may not be protoplasm is a question the answer to which lies not in the realm of morphology but of energetics. It may well be that it is to the action of the lipoidal substances and soaps that we may look hopefully for the solution of some of the anomalies in permeability.

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[1] *Ber. d. Deut. bot. Gesell.*, 1919, xxxvii, 380.

The hydration reactions of living cell masses, of dried tissues and of plates of colloids simulating the composition of living matter having been determined, attention was next turned to experiments which might afford a means of estimation of the action of such material in the vacuolated cell. In brief it was found necessary to devise a new or improved form of a little used type of artificial cell. It was seen that such a device should be one in which the character of the wall might be changed, the plasma represented by various jellies with consequent variations in the contact zones (phase boundaries), and furthermore that such a cell could be subjected to a range of solutions, externally and internally comparable to those of the living cell. Such a cell consists essentially of a capsule of a gel enclosed in walls of varying porosity and other mechanical characters. To facilitate determinations of action the capsule must be fitted with an osmometer head for measurement of volume or pressure. The experiments which have been made to this date have been with cells in which agar, gelatine and various mixtures of these with potassium oleate, calcium oleate, lecithin and other substances have been used as the "plasmatic" layer and the outer wall was represented by platinum gauze, Whatman's double thickness filter- or extraction-thimbles, wooden cups, and by clay cups such as are used in the Livingston atmometer. The ranges of conditions offered for the study of absorption and of osmose are fairly equivalent to those of the living cell.

If we now take such a cell and set it in operation to ascertain what may happen when the vacuoles begin to appear by syneresis in the protoplasm, using for example one in which the jelly forms a layer about 3 mm. in thickness on the inner side of the wall with a cell content of 30 to 40 c.c. we may get what may be taken to be the initial action in such a case by filling it with water and setting it in water. The first thing to occur in such a case will be the hydration of the jelly to a point approaching its maximum. Coincidently the solution or dispersion of material from the inner surface of the layer begins in a manner which should be practically identical with that which ensues with the enlargement of the vacuoles. The action of these free particles of colloidal material results in a mounting osmose which measured by the exudation from the outlet of the cell reaches its maximum between 75 and 100 hours at 15° C., at about 3.5 to 4 c.c. daily and, when measured in terms of pressure set up by a cell lined with agar the magnitudes are in a range not far from those given for gum acacia. This secretion or exudation was found to continue for as long as 70 or 80 days in cells with a plasmatic lining of a mixture of agar, gelatine and potassium oleate, during which time the total excretion would amount to two and a half times that of the capacity of the cell, that is about 75 or 80 c.c. The dispersion and osmotic action of the plasmatic colloids in the vacuoles as illustrated must be recognized as one of the initial factors in the distention and turgidity of the cell. Diffusion of sugars and their decomposition products, and of amino-compounds of various kinds into the vacuole, must also ensue from a constantly renewed supply furnished by the metabolic activity of the protoplasm. Salts included in the plasma must also be diffused into the vacuolar liquid, and it is to electrolytes that Pfeffer and others attribute as much as sixty per cent. of the total osmotic capacity of the cell, which in the higher plants shows a potential from 3 to as high as 150 atmospheres as determined by J. A. Harris and others using cryoscopic and plasmolytic methods. When it is realized that the partial pressures which must be attributed to the electrolytes are much higher than those which are ordinarily found in soil solutions or in media in which simple organisms live the manner in which the higher concentrations are accumulated in the cell becomes as much of a problem as that offered by glandular action in the body of the animal.

Some measurements of cumulative action in building up turgidity have been made, in which it was shown that a cell mass which might be plasmolyzed at a certain high concentration if first immersed in a series at lower concentrations might be led up to endurance of this concentration without loss of tugidity. A similar action may be demonstrated by the artificial cell described above. When lined with an agar-gelatine-potassium oleate mixture it shows a tonicity of about 0.00 M. potassium chloride, showing no positive action when filled with water and immersed in a concentration above this. If however it is first allowed to get into full action in the above concentration it may then by small steps be moved up to where it shows a tonicity equivalent to 0.005 M. This is a short series of small steps, but it is one shown by a cell in a static condition in the sense that none of its parts are being renewed or altered by metabolism, but most important of all it is in the direction of cumulative turgidity. Such action may or may not however imply any accumulation of electrolytes but it is proven that the cell contents by changes in material partly or entirely derived from the jelly or plasma shows an increasing osmotic pressure measured against potassium chloride. The actual diffusion of the salt into the vacuole has not been measured.

It is the external zone or phase boundary of the plasmatic layer to which attention has been chiefly directed in studies of permeability and diffusion of salts into and out of the cell. Its original structure at the moment of formation of the new cell is in all probability that which might be predicated for the external layer of any such a semi-liquid mass of jelly, composed of albumens, pentosans, soaps and lipoids, the two last-named forming but a small proportion of the dry weight of the mass, but by their surface tension relations would tend to assume a peripheral position. Following this initial arrangement it is also to be seen that compounds of these groups would continue to be added from the products of a continuing metabolism. The resulting formation would present a picture widely different from that of the parchment membrane to which it has been so often compared. In accordance with this view the shrinkage of the protoplasm from the wall in plasmolysis is not accompanied by a detachment of a membrane from the wall but by an actual rupture or partial destruction of the peripheral layer. When in addition my own results to the effect that the hydration or swelling of dried sections of biocolloids is accelerated by balanced solutions of sodium, while the osmotic action of artificial cells lined with the same biocolloids is seen to be not affected by antagonisms, a revision of some widely prevalent views as to the nature of permeability seems to be required.

If we now turn attention to the outer wall of the vegetable cell we see it developing from a semi-liquid cell-plate to a condition approaching that of densely layered and infiltrated structure;

from a condition of extreme permeability to one in which solutions of common salts may not pass through it. It is of course to be recalled that not only does the wall change with its growth but that it may do so quite irregularly so that the wall may have impermeable areas and others of ready permeability.

Some possibilities of the influence of the wall on the exchange between the protoplasm and its environment are suggested by effects in negative osmose which have been obtained by the use of the artificial cell, this term *negative osmose* being used to denote increase in volume of the solvent in the cell against a solution of higher concentration external to the cell. Thus when an osmometer comprising only the outer clay or wooden wall of the artificial cell is filled with water and immersed in a solution of calcium chloride at various concentrations ranging from 0.0035 M. upward negative action ensues, the contents increase in volume and exudation takes place. If the cell be given its artificial plasmatic lining the effect continues, overbalancing the positive osmotic action of the colloidal components dissolved in the cell. If the electrolyte be placed in the cell its action dominates and exosmosis, being "negative" osmose, ensues. These results so far as they have been examined being consonant with those of Bartell, who has recently made many measurements of negative osmose through porcelain plates.

A summarization of the facts and conclusions cited in this paper may be made as follows:

1. All substances which may appear in the cell in the colloidal condition of reversible gels must be taken into account in any adequate interpretation of cell-action, particularly growth. Albumin, pentosans, soaps and lipins are thus to be taken into consideration in cell-mechanics. The findings of Hansteen-Cranner to the effect that lipins accumulate in quantity at the periphery of the protoplast and run in a meshwork from cell to cell and through the cell does not justify his conclusion that such material is the ground substance of living matter. The actual identity of living matter is not a question of structure but is one of integrated action, not one of equilibria but one of energetics.

2. All attempts to find separable membranes to protoplasts by dissection, and by microscopic and ultramicroscopic methods have

ended negatively. The so-called membranes are peripheral layers resulting from the action of surface tension in masses of widely varying composition under a wide range of external conditions.

3. All of the substances known to promote growth which have been tested have been found to accelerate hydration in living cells, dead cell-masses and protein-pentosan,—soap jellies at particularized concentrations from 0.01 M. to 0.000,01 M. and through a range of $P_H$ from 4 to 11. Such action would represent the action of young cells. The second phase of growth of the cell is accompanied by the syneretic formation of vacuoles setting up conditions, which have hitherto been interpreted in terms of osmotic equilibria.

4. The demonstration of the fact that polarizable separable membranes are not formed, that the peripheral layer is not dominantly proteinaceous, and that osmotic potentials are accumulated in the cell far overbalancing that of the medium makes evident the necessity for some new interpretations.

5. The attempt is recorded to make up and operate an artificial cell the outer wall of which should be a fixed colloid of clay, wood, or parchment, and a lining or plasmatic layer of reversible gel, simulating protoplasm, with a view to obtaining information as to the accumulation of electrolytes in the cell with resultant overbalancing osmotic potentials.

6. Such cells filled with water and immersed in water show an intake and excretion for a continuous period of 60 to 80 days at 15° C. during which time the vacuolar content of the cell is replaced two or three times.

7. The artificial cell may be arranged to show exosmose in its earlier stage, followed by endosmose, and negative osmose by the action of calcium or magnesium salts as vacuolar contents or as immersion media. Cells which have a tonicity equivalent to 0.003 M. potassium chloride when filled with water may be raised to a tonicity of 0.005 M. by immersion in a series of increasing concentrations, after a manner which has been used in raising the tonicity of living cell-masses.

8. The course of metabolism, the action of light and the transpiration of water may be held to account for the concentration of carbohydrates and electrolytes and the conversion of sugars

and proteins to more highly osmotically active forms in leaves and other organs. There occurs however in many tracts of the plant an accumulation of material, in which diffusion or excretion may be said to work against osmosis after the general manner illustrated by glandular action in animals. The use of the artificial cell promises results of interest in the solution of such problems.

53 (1800)

## The bacterial content of the stomach as influenced by saliva.

By **NICHOLAS KOPELOFF.**

[*From the Department of Bacteriology of the Psychiatric Institute, Ward's Island, New York City.*]

The fractional method of gastric analysis makes possible a bacteriological study of the stomach which includes not only the active cycle of digestion, but the resting phase as well. So far as could be ascertained, no quantitative bacterial studies employing this method have heretofore been reported. In fact, very little data concerning the types of bacteria in the stomach at different stages of digestion have appeared in the literature beyond the work of Cotton.[1]

In a previous paper,[2] the writer has shown that repeated analyses on the same individual within a short period of time—while the physical and mental condition remain practically unchanged—yield different curves. These curves from the same individual often vary as much from one another as the difference between the curves of different individuals. This holds true likewise for the average fasting contents. Therefore the conclusion was reached that single determinations by the Rehfuss method are not sufficient upon which to base valid conclusions since they do not take into consideration individual variations. In bacteriologic studies of fractional gastric analyses carried out repeatedly on psychotic patients and normal individuals, in only one half the instances was there any correlation between high acidity and low bacterial numbers or vice-versa. Upon close observation it became apparent that the amount of saliva swallowed by a patient during the two and one half hour period necessary for the complete gastric analysis by the fractional method, was of considerable significance. Only one patient, a case of profound depression, consistently showed a complete absence of bacteria on repeated analyses. Her mouth was usually exceedingly dry. The conclusion, therefore, was that the absence of saliva was the limiting factor, so far as her bacterial content was concerned.

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[1] Cotton, H. A., *N. Y. Med. Journ.*, 1920, cxi, 672–677; 721–727; 770–775.

[2] Kopeloff, Nicholas, read before the Brooklyn Neurological Society, October 19, 1921 (in press).

It is manifestly impossible to completely prevent the swallowing of all saliva during the course of a fractional gastric analysis. The method finally devised, however, proved effective in reducing the swallowing of saliva to a minimum. The procedure consisted in placing an ordinary dental suction tube, which was attached to a running water vacuum pump, in the subject's mouth throughout the analysis. One c.c. of each fraction was plated in triplicate at once upon withdrawal, on both glucose meat infusion agar and lactose meat infusion agar to which brom cresol purple was added.

| M. Sl. | Saliva Not Removed | | | Saliva Not Removed | | | Saliva Removed | | |
|---|---|---|---|---|---|---|---|---|---|
| | Bact. Per 1 c.c. | Total Acidity | $P_H$ | Bact. Per 1 c.c. | Total Acidity | $P_H$ | Bact. Per 1 c.c. | Total Acidity | $P_H$ |
| F.C... | 15,500 | 5 | 2.8 | | | | 2 | 12 | 2.8 |
| ¼ HR. | 380 | 11 | 2.7 | 310 | 35 | 2.9 | 5 | 23 | 2.5 |
| ½..... | 78 | 41 | 2.2 | 5,100 | 37 | 3.0 | 8 | 52 | 1.7 |
| ¾..... | 5 | 46 | 1.8 | 925 | 41 | 2.8 | 2 | 70 | 1.4 |
| 1..... | 60 | 25 | 2.5 | 2,800 | 28 | 3.0 | 0 | 71 | 1.3 |
| 1¼.... | 800 | 18 | 2.7 | 110 | 36 | 2.2 | 1 | 42 | 1.5 |
| 1½.... | 215 | 25 | 2.3 | 95 | 43 | 1.9 | 1 | 29 | 1.7 |
| 1¾.... | 55 | 6 | 2.7 | 12 | 45 | 1.8 | 32 | 12 | 2.0 |
| 2..... | 110 | 28 | 2.7 | 5,400 | 10 | 3.7 | * | 2 | 3.0 |
| 2¼.... | 48,000 | 9 | 2.7 | 3,200 | 9 | 4.3 | * | | |
| 2½.... | 46,000 | 10 | 2.7 | 6,800 | 16 | 3.5 | * | | |

In the table are presented the bacteriological and chemical results obtained with a psychotic patient (diagnosed manic-depressive: manic). Contrast the first two columns of bacterial numbers where saliva was not removed with the column of bacterial figures where saliva *was removed*. In the latter instance the first number is 2 and the last, which is the highest, is 32. Such a striking reduction makes the conclusion irresistible: namely, that bacterial numbers in the stomach at any one time depend almost entirely upon the saliva swallowed (where the bacterial content of the food may be disregarded). It would be expected that the greatest multiplication of bacteria and maximum numbers would be attained in the "interdigestive" phase, when the stomach is relatively at rest and the secretion of acid is at a minimum. Accordingly, therefore, the fasting contents should show the highest bacterial count. But such is not the case. As a matter of fact the last fractions, whether saliva be removed or not, contain a far greater number of bacteria, and this is additional evidence that the continual swallowing of saliva (which contains millions of bacteria per c.c.) is in reality the factor which determines the bacterial content of the stomach at such a time. Again, the fact that the bacterial numbers where saliva was removed, were so small as to be negligible is significant when it is noted that the secretion of acid is without much influence, *i.e.*, only 2 bacteria appear where the total acidity is as low as 12 and as high as 70. All these considerations mentioned point to the fact that the saliva is the most important single factor in influencing the bacterial content of the stomach under the conditions employed. Similar results were obtained when these tests were made on a healthy normal individual and on other manic patients having very low acidity. The most important consideration, however, is that these patients having a very low acidity would be precisely the type of subjects, therefore, in which bacteria might gain a foothold and make the stomach a focus of infection. Judging from the results when saliva is removed, such is far from being the case.

A qualitative study of the types of bacteria found in the saliva, and by the fractional method of analysis, in the stomach, serves as additional evidence in support of these findings. Consequently, when the Rehfuss method is employed, the stomach cannot be considered a focus of infection except where lesions are known to exist.

### 54 (1801)

## The effect of the accessory substances of plant tissue upon growth of bacteria.

By O. T. AVERY and HUGH J. MORGAN.

[*From the Hospital of The Rockefeller Institute for Medical Research, New York City.*]

The study of the properties of blood upon which depends the ability of the so-called hemoglobinophilic bacilli to grow in this medium, has shown that these properties are related to at least two factors which can be separately studied. Further studies have shown that both of these factors are present in plant tissue, (potato and banana), and that sterile unheated vegetable tissue can replace blood in the cultivation of *B. influenzæ.* These observations have now been extended; yellow and white turnip, carrot, beet, parsnip, and sweet potato, when added to fluid media have been found to possess the same growth stimulating action as white potato.

It has been found that these vegetable tissues not only permit the cultivation of the so-called hemoglobinophilic organisms, but that they also greatly favor the growth of other entirely unrelated organisms. For instance, in the case of pneumococcus, not only is there a marked acceleration of growth, but a seeding too minute to initiate growth in plain broth alone, will amply suffice to induce abundant multiplication in the same medium to which small pieces of sterile, unheated vegetable have been added. Moreover in the plant tissue medium the zone of hydrogen-ion concentration within which growth can be initiated is considerably extended beyond the acid and alkaline limits of the optimal range in ordinary bouillon. In addition certain other bacteria, which ordinarily fail to grow in the presence of free oxygen, multiply in a medium containing fresh plant tissue even though no precautions are taken to exclude air. It is evident, therefore, that the presence in media of certain substances contained in fresh plant tissue not only supplies the necessary factors for growth of the hemoglobinophilic bacilli, but furnishes the requisite requirements for the cultivation of other bacteria which multiply only under certain restricted conditions. One such condition is the reaction of the medium which in the presence of plant tissue may be made to vary over a much wider zone without retarding growth; another condition is oxygen tension which similarly seems to require for sensitive organisms much less accurate control in the presence of plant tissue than in its absence.

The exact nature of the substances contained in plant tissue upon which these properties depend is not yet determined, but the studies so far made suggest that they are related to the presence of certain oxidizing and reducing enzymes in fresh plant tissues as well as to the presence of so-called accessory food substances.

### 55 (1802)

## The diffusion of sodium chloride through a "lecithin"-collodion membrane.

By **HAROLD A. ABRAMSON** and **SAMUEL H. GRAY.**

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

A study of the diffusion of sodium chloride through: (*a*) membranes prepared from collodion; and (*b*) membranes prepared from collodion which contained approximately four grams of commercial "lecithin from eggs" per hundred cubic centimeters, was made under the following conditions.

1. The collodion (Eimer and Amend's, U.S.P. IX) contained about four grams of guncotton per hundred cubic centimeters. The membranes, therefore, which contained "Lecithin" were approximately fifty per cent. lipoid by weight.

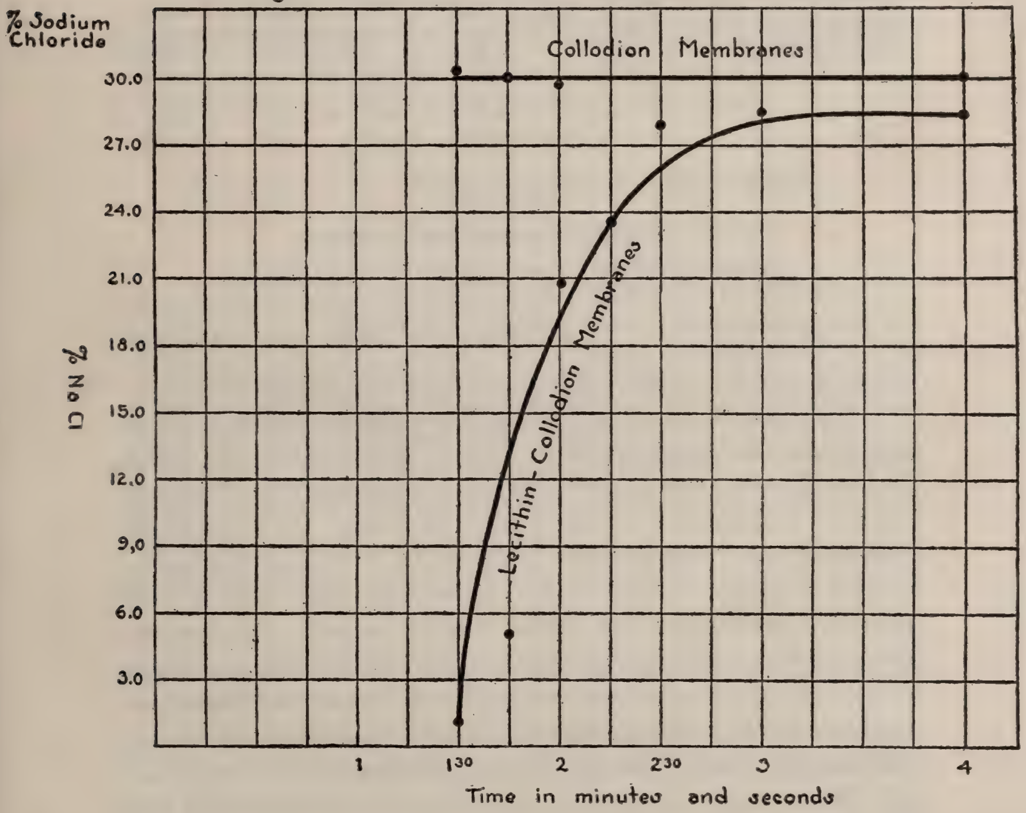
2. The membranes were made with as nearly uniform technique as possible. The viscousness of the collodion and of the lecithin-collodion solution was kept constant and the same. The volume of the membranes, which were shaped inside of Erlenmeyer flasks, varied between fifty and sixty cubic centimeters.

3. The only factor experimentally varied was the drying time which was terminated by fixation with tap-water.

4. In every determination of permeability, the membranes were filled with one fourth molar sodium chloride and immersed

in beakers containing three hundred and fifty cubic centimeters of water. At the end of twenty minutes they were withdrawn and a known volume of the outside fluid titrated for chloride.

Average Diffusion from Tables I and II



The following tables enumerate the results of one set of a series of experiments. The drying time of one minute and thirty seconds was the lowest at which membranes which did not cloud could be prepared.

The least permeable fixed lecithin-collodion membranes were clear. Those of the later drying times were delicately opalescent. This opalescence was more marked when the drying time was increased beyond four minutes. A study of the tables shows that with the change from clearness to opalescence there was a corresponding change in the permeability of the membranes. The pure

### TABLE I.

### COLLODION MEMBRANES.

*Percentage of Sodium Chloride Diffusion in Twenty Minutes.*

| Drying Time, (Minutes and Seconds). | 1:30 | 1:45 | 2:00 | 4:00 |
|---|---|---|---|---|
| Membrane No. 1 | 30% | 30% | 30% | 29% |
| 2 | 31% | 30% | 30% | 30% |
| 3 | 30% | 29% | 28% | 28% |

### TABLE II.

### "LECITHIN"-COLLODION MEMBRANES.

*Percentage of Sodium Chloride Diffusion in Twenty Minutes.*

| Drying Time, (Minutes and Seconds). | 1:30 | 1:45 | 2:00 | 2:15 | 2:30 | 3:00 | 4:00 |
|---|---|---|---|---|---|---|---|
| Membrane No. 1 | <2% | 1% | 25% | 21% | 30% | 27% | 28% |
| 2 | 1% | 6% | 27% | 11% | 29% | 30% | 28% |
| 3 | <1% | <1% | 24% | 27% | 28% | 28% | 29% |
| 4 | 3% | 12% | 3% | 28% | 28% | 28% | 29% |
| 5 |  | 8% |  |  | 28% | 28% | 26% |

collodion membranes were all clear and had a practically constant diffusion rate. This is suggestive of the possibility that the change in the size of the aggregates of lecithin molecules may have here influenced the rate of passage of the salt. Neither structure nor fat-droplets were visible under the oil-immersion of stained and unstained sections. In this connection it is interesting to note that membranes prepared with olive oil become definitely cloudy in less than one minute before fixation and this cloudiness persists after fixation. In these membranes definite oil droplets are visible under low power. Curiously enough both olive oil and lecithin-collodion membranes are clear after fixation when the drying time was extended over hours. The study of these membranes is incomplete.

## CONCLUSIONS.

I. Changes in the drying time, to four minutes, of membranes prepared from collodion and fixed with water does not appreciably alter their permeability to sodium chloride, whereas membranes prepared under the same conditions containing fifty per cent. of "Lecithin" by weight become relatively semi-permeable with decreasing drying times.

II. Changes in the sizes of the aggregates of the lecithin molecules is suggested as a possible influence on the permeability of the lecithin-collodion membranes.

### 56 (1803)

## Notes on studies in the physiology of the gall bladder.

By **LIONEL S. AUSTER** and **BURRILL B. CROHN.**

[*From the Laboratory of Physiology, Cornell University Medical College, New York City.*]

In undertaking the following animal experiments on the nature of the expulsive action of the gall bladder, we had particularly in mind the investigation of the modus operandi of magnesium sulphate when applied to the papilla of Vater as suggested by Meltzer.

Laparotomy and duodenotomy was performed on several dogs anesthetized with chloretone. Observation of the gall bladder immediately after laparotomy showed a distended bladder in all except one animal. The flaccid bladder was seen in a fasting dog.

A solution of methylene blue was injected into the gall bladder to differentiate its content from the bile flowing from the liver. The duodenal mucosa in the region of and including the papilla of Vater was irrigated with magnesium sulphate solution; although an increased flow of bile was observed, no expulsion of the gall bladder content was noted. This observation was carried on for several hours in a series of eight dogs. The gall bladder retained its bile independent of whether the dog was in the fasting or the actively digesting state.

Stimulation of liver bile flow as obtained with magnesium sulphate was also observed after the application of sodium sulphate, sodium phosphate, peptone, $N/10$ hydrochloric acid, bile and sodium glycocholate. No stimulation was seen after the application of water, sodium chloride or sodium hydroxide.

Attempts to produce contraction by nervous stimulation were unsuccessful. Strong direct faradic stimulation of the organ failed to produce contraction or expulsion of contents.

A series of experiments, in which phenoltetrachlorphthalein was injected intravenously and subsequently recovered when excreted in the bile, showed the appearance of this substance in the duodenum in from ten to fifteen minutes after injection. A flow into the gall bladder of liver bile was demonstrated by the recovery of the phenoltetrachlorphthalein from the bladder bile when the cystic duct was patent. None of the dye was found in the sac when the duct was tied off.

Observations on the filling of gall bladders emptied by digital compression showed the filling to be a slow and irregular process in spite of the fact that the bile flow into the duodenum may or may not be continuous. Bile flow into the duodenum was observed with regularity in the cases of fasting dogs as also in the absence of immediate digestion.

Stasis of gall bladder contents was investigated in a series of experiments in which, under aseptic precautions, a sterilized suspension of an inert finely divided substance (charcoal or carmine) was introduced into the gall bladder. The dogs were permitted to live for periods of 12 hours, 1, 3, 7 days. During these periods they were fed a mixed ration of protein, fat, carbohydrate and water. At autopsy, the coloring matter was recovered from the gall bladders up to and including three days. No traces were found in intestinal washings. The bladder was found to have emptied in one week. Patency of the ducts was shown by the slight digital pressure needed to expel the bladder contents.

57 (1804)

## The destruction of the antiscorbutic vitamin in milk by the catalytic action of minute amounts of copper.

By **ALFRED F. HESS** and **LESTER J. UNGER.**

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

In a previous communication[1] it was shown that the antiscorbutic vitamin in milk, orange juice, or tomato juice, is, to a certain extent, destroyed by oxidation. In view of the well-known action of catalysts in increasing oxidative processes, and the frequent presence of traces of copper in milk, it seemed worth while to ascertain the effect of the addition of small amounts of copper to milk. To this end two groups of guinea pigs were fed equal amounts of milk, which, in the one instance, had been heated in a glass vessel to 60° C. for 40 minutes, and, in the other instance, had been heated in a copper vessel to the same degree. Each animal received daily dried milk to the equivalent of 100 c.c. of fluid milk, and oats in addition. Diluted dry milk was employed, so as to be able to concentrate the milk and thus insure its complete consumption.

None of the guinea pigs fed on the milk heated in the glass vessel developed scurvy during a period of four months; they did not, however, make normal gains as the quota of antiscorbutic vitamin was inadequate. On the other hand, the animals fed with the same quantity of milk, which had been heated in a copper vessel, all developed scurvy, and died after about four weeks. This milk contained 1.4 part of copper per million. That this nutritional failure was due to a lack of antiscorbutic vitamin was demonstrated by the excellent gains of a third group of guinea pigs which were given milk which had been heated in the copper vessel, but received in addition 2 c.c. daily of orange juice.

Milk frequently becomes contaminated with copper in the course of commercial pasteurization or condensation. It seems possible that these traces of copper may contribute to the destruction fo the antiscorbutic, vitamin and account, in some degree, for the varying content of this factor in heated milk. It is also possible that the copper ingested with the food, especially in infants whose diet consists mainly of milk, may exert an effect within the animal body.

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[1] Hess, A. F. and Unger, L. J., PROC. SOC. EXPER. BIOL. AND MED., 1921, xviii, 143.

### 58 (1805)

## The prevention of rickets in the rat by means of radiation with the mercury vapor quartz lamp.

By G. F. POWERS, E. A. PARK, P. G. SHIPLEY, E. V. MCCOLLUM and NINA SIMMONDS.

[*From the Department of Pediatrics, Yale University, New Haven, Conn., the Department of Pediatrics, Johns Hopkins University, Baltimore, Md., and the School of Hygiene, Johns Hopkins University, Baltimore, Md.*]

All the evidence as to the preventive and curative effects in the rickets of human beings of the radiations from the mercury vapor quartz lamp has been furnished by the X-ray. In order to determine the protective action of these radiations in experimental rickets in rats and also to examine the bones themselves we performed the following experiments.

Nineteen rats, mostly mixed black and white and about seven weeks old, were placed on diet 3143 which, as previous experience has shown, produces rickets comparable in every respect to the rickets manifesting itself in human beings.

Nine rats were kept as control animals under ordinary laboratory conditions in a room completely screened with windows of ordinary glass (cage "R" animals). Ten rats were exposed to the radiation from a Hanovia mercury vapor quartz lamp (Alpine type) (cage "U–V" animals). One animal (16Y) in cage "R" was found paralyzed thirty-eight days after being placed on diet (age about eighty-eight days) and was killed. We have previously pointed out that the development of paralysis of the posterior extremities not infrequently occurs in rats fed on diet 3143. Another animal (26Y) was killed fifty-eight days after being placed on the diet (age about one hundred and eight days); and the other seven animals were killed sixty-four days after being placed on the diet (age about one hundred and fourteen days). The animals in cage "U–V" (rayed animals) were exposed to the radiations at a distance of three feet for varying periods of time daily for sixty-four days and were then killed.

The rayed animals as contrasted with the control animals showed marked physical vigor as evidenced by growth, activity, good appetite, thick smooth coats and reproductive power.

Autopsies showed the rayed animals to be larger than the controls and to have great increase over the controls in the amount of fat deposition and muscular development. The rayed animals showed no evidence of rickets. The control animals showed enlargement of the epiphyses of the long bones, deformities of the thorax, enlargement of the costo-chondral junction and fractures of the ribs. Histological examination showed the long bones of the rayed animals to be normal and those of the control animals to have typical rickets.

The effects of the radiations of the mercury vapor quartz lamp on the growth and calcification of the skeleton of the rat and on the animal as a whole seem to be similar if not identical with those brought about by direct sunlight and by cod liver oil.

### 59 (1806)

## Collodion sacs for aërobic and anaërobic bacterial cultivation.

By FREDERICK L. GATES.

[*From the Rockefeller Institute for Medical Research, New York City.*]

The collodion sacs demonstrated before this society a year ago,[1] while suitable for intraperitoneal implantation are not so well adapted to microbic cultivation *in vitro*. We have therefore been making sacs of 5–10 c.c. capacity in test tubes lined with a dried film of gelatin[2] which softens in warm water and permits the easy removal of the collodion membrane. The sac is slipped on to a supporting glass tube, inserted into one limb of a V-shaped tube, open at both ends, and sealed in place with a collar of rubber tubing. Sac and V tube are partly filled with water, plugged with cotton and sterilized in the autoclave. Shrinkage during sterilization may be avoided by maintaining a pressure of 10–12 cm. of water in the sac. The sac may even be expanded by this method, but its permeability apparently is not thereby increased.

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[1] PROC. SOC. EXPER. BIOL. AND MED., 1920, xviii, 92. *Jour. Exper. Med.*, 1921, xxxiii, 25.

[2] The 10 per cent. gelatin solution is preserved with 0.3 per cent. tricresol.

After sterilization the chosen medium is placed within the sac, and dialysis of nutritive and growth-promoting substances occurs into the surrounding fluid, which is accessible for inoculation through the other limb of the V tube. For anaërobic cultivation both medium and dialysate may be layered with vaseline. The vaseline seal excludes oxygen, but also retains $CO_2$ and may therefore tend to a more rapid acidification of the medium.

Osmotic pressure adjustments take place automatically by changes in the levels of medium and dialysate. With experience the approximate osmotic pressure of a given medium may be anticipated and the passage of water into the sac may be avoided by filling it with medium to a higher level.

These sacs were prepared especially for use with the Smith-Noguchi fresh tissue medium. This medium, consisting of ascitic fluid or dilute serum and a fragment of fresh rabbit kidney or testicle is placed within the sac, in the dialysate of which we have grown subplants of *T. pallidum*, *S. microdentium* and *Bacterium pneumosintes*. Thus the organisms have been obtained free from the confusing, antigenic, protein precipitate which develops in the tissue medium. The addition of dextrose broth hastens the establishment of anaërobic conditions.

For mass cultures the sacs have been formed in gelatin-lined Erlenmeyer flasks, and enclosed in larger flasks with a glass tube, or spout, fused into the side near the bottom.

A full description of the sacs will appear in a forthcoming number of the *Journal of Experimental Medicine.*

60 (1807)

## A delicate biological test for calcium-depositing substances.

By E. V. McCOLLUM, NINA SIMMONDS, P. G. SHIPLEY, and E. A. PARK.

[*From the Department of Chemical Hygiene, School of Hygiene and Public Health, and from the Department of Pediatrics, Johns Hopkins University, Baltimore, Md.*]

Some time ago we described two diets, Nos. 2638 and 2677, which we used with a view to the development of a biological test which would show the calcium-depositing power of any given substance.[1] The test was carried out as follows: The faulty diet was first fed to a group of young rats for the purpose of making the epiphyseal cartilage free from calcium, and producing a rachitic metaphysis. After a sufficiently long period had elapsed, the test substance was added to the diet of those animals which were to serve as test subjects, while the faulty diet without the test substance was continued in the case of the control rats. Substances, which when added to the faulty diets enabled the organism to deposit lime salts, caused the reappearance of the provisional zone of calcification in the bones. This biological test we called the "line test," because the new provisional zone of calcification appeared as a line of calcium salts extending transversely across the bone with a limeless cartilage on one side of it and a limeless metaphysis on the other.

The success of this test depends on the use of a diet which uniformly causes the epiphyseal cartilage and the metaphysis to be free from calcium salts. It is not sufficient that a diet should merely produce rickets. The rickets which it produces must be of so severe a type that no vestige of calcium remains in the cartilage, and a wide metaphysis is formed. Moreover, the diet must be so constituted that the animals restricted to it will grow and maintain a fair state of general health and nutrition. The diets which we earlier described were not satisfactory, since they did not invariably produce typical rickets. Now and again an animal which was restricted to one of them would be found whose cartilages contained deposits of calcium salts. We have finally worked out a diet which fulfills the requirements for the test.[1]

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[1] Shipley, P. G., Park, E. A., McCollum, E. V., Simmonds, Nina, and Parsons, H. T. *Jour. Biol. Chem.*, 1921, xlv, 343.

DIET (LOT) 3143.

| | |
|---|---|
| Wheat | 33.0 |
| Maize | 33.0 |
| Gelatin | 15.0 |
| Wheat gluten | 15.0 |
| NaCl | 1.0 |
| $CaCO_3$ | 3.0 |

This diet is faulty because it contains only 0.3019 gram of phosphorus in 100 grams of the mixture, while calcium is present to the extent of 1.221 grams per 100 grams of food. We have found that about 0.40 gram of phosphorus per 100 grams of food represents the lowest concentration of this element on which optimal nutrition can be secured when 0.641 gram of calcium are present in each 100 grams of ration. The fat-soluble A is low in this diet (Lot 3143), but it is sufficient to allow growth and to protect the rat against xerophthalmia. The diet also contains very little of an uncharacterized organic substance present in certain fats. We have previously discussed in detail the results of restricting rats to it.

## TECHNIC OF TEST.

A group of young rats is placed on diet 3143 for 35–45 days, or until they begin to lose control of their hind legs. They are then divided into two groups, a control group which continues to receive diet 3143 unchanged, and a test group, which is given for the number of days deemed necessary the faulty diet plus the substance which is to be tested.

When a sufficient number of days have elapsed for the test substance to have produced its effects, the animals are killed and the bones which are to be studied are split longitudinally. The proximal end of the tibia is best for the purpose. One half of the bone is immersed in a dilute silver nitrate solution and exposed to light. It is then examined in the solution through a binocular microscope for the presence of a newly formed line of calcification in the proliferative cartilage. This line of calcium, which looks like the cross section of a honeycomb under strong magnification, is blackened by exposure to light. If this line is present, the test is positive. The line may be visible to the unaided eye in untreated bones. The results of the examination of the gross specimen should be confirmed by study of celloidin or frozen sections from the other half of the bone or other bones. Control rats and rats which fail to give the test do not show the line of calcification. The new line of calcification may extend completely across the bone or may be incomplete or fragmentary, according to the extent of the deposition of the lime salts induced by the substance which is under examination. Since complete starvation also causes the typical linear deposit of lime salts to appear in the cartilage of rachitic animals, the food intake of both test and control rats must be carefully watched during the course of the experiment. Control and test animals must be kept under identical conditions.

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[1] Shipley, P. G., Park, A. E., McCollum, E. V., and Simmonds, Nina. PROC. SOC. EXPER. BIOL. AND MED., 1921, xviii, 277.

This method is applicable to the study of the calcium depositing-power of any chemical substance or physical force.

### 61 (1808)

## The effects of pituitary extract on the body temperature of animals rendered poikilothermous by destruction of the optic thalamus.

By FRED T. ROGERS.

[*From the Department of Physiology, Baylor Medical College, Dallas, Texas.*]

In earlier work the writer has shown that destruction of the cerebral hemispheres and the optic thalamus of birds reduces the animal permanently to a poikilothermous condition. In birds this is not an operation that leads to immediate death for they may be kept alive for one to three months by keeping them constantly at an atmospheric temperature of 30° to 35° C. The routine procedure was to remove the cerebral hemispheres in toto by the scalpel and then destroy the optic thalamus with an electro-cautery.

It has been pointed out elsewhere that to produce the poikilothermous condition there must be extensive destruction of the thalamus and that localized injuries did not appreciably change the body temperature regulation. This destruction of the structures around the third ventricle, it is obvious, might also involve the hypophysis. Inasmuch as a subnormal temperature is among the cycle of disturbances following injuries or removal of the hypophysis (Cushing and others) the temperature disturbance might be attributed to hypophyseal injury, rather than to the lesion in the brain. The experiments have been repeated therefore taking particular care not to traumatize the hypophysis.

A series of pigeons were reduced to the poikilothermous condition by cauterization of the thalamus. After death absence of any gross visible changes in the hypophysis was confirmed. The organ in each of these animals had a perfectly normal appearance although there may have been circulatory alterations or cytological changes invisible to the naked eye.

However this may be the injection intra-peritoneally of from .2 to 1.0 c.c. of pituitary extract (posterior lobe—Lilly) causes a sharp rise in body temperature of the poikilothermous birds.

TABLE I.

POIKILOTHERMOUS DECEREBRATE PIGEON.

| Date. | Time. | Temperature of Cage. | Body Temperature. |
|---|---|---|---|
| Nov. 3...... | 8.00 A.M. | 25° C. | 31.2° C. |
| " "...... | 11.00 A.M. | 25° C. | 30.4° C. |
| " "...... | 12.30 P.M. | Injection of .4 c.c. of pituitrin | |
| " "...... | 2.00 P.M. | 25° C. | 34.2° C. |
| " "...... | 3.30 P.M. | 25° C. | 36.0° C. |
| " "...... | 5.00 P.M. | 26° C. | 38.1° C. |
| " "...... | 8.00 P.M. | 26° C. | 37.0° C. |
| " "...... | 11.30 P.M. | 26° C. | 36.2° C. |
| Nov. 4...... | 10.00 A.M. | 25° C. | 34.8° C. |
| " "...... | 2.00 P.M. | 25° C. | 34.6° C. |
| " "...... | 2.15 P.M. | Injection of .4 c.c. of pituitrin | |
| " "...... | 3.15 P.M. | 24° C. | 37.0° C. |
| " "...... | 4.30 P.M. | 24° C. | 38.0° C. |
| " "...... | 6.00 P.M. | 24° C. | 39.1° C. |
| " "...... | 7.30 P.M. | 24° C. | 39.3° C. |
| Nov. 5...... | 10.00 A.M. | 25° C. | 32.0° C. |

Injection of the extract into normal birds causes no temperature reaction greater than the range of the diurnal variations.

In the poikilothermous pigeon whose body temperature is artificially maintained at a normal level by keeping in a warm incubator, injection of pituitary extract is followed by a rise in temperature such as to threaten heat prostration.

TABLE II.

POIKILOTHERMOUS DECEREBRATE PIGEON.

| Date. | Time. | Temperature of Cage. | Body Temperature. |
|---|---|---|---|
| Nov. 6...... | 12.15 P.M. | 30° C. | 40.2° C. |
| " "...... | 2.00 P.M. | 30° C. | 39.0° C. |
| " "...... | 4.00 P.M. | 30° C. | 40.0° C. |
| " "...... | 4.15 P.M. | .4 c.c. of pituitrin | |
| " "...... | 5.30 P.M. | 30° C. | 43.1° C. |
| " "...... | 6.00 P.M. | 30° C. | 43.2° C. |
| " "...... | 7.30 P.M. | 30° C. | 43.5° C. |
| " "...... | 9.00 P.M. | 24° C. | —— |
| Nov. 7...... | 10.00 A.M. | 24° C. | 32.0° C. |

The rise in body temperature persists for twelve to twenty-four hours and then falls to a level determined by the environmental temperature.

We have been unable to continuously maintain the body temperature at the normal level by pituitary extract alone without the aid of the warm incubator. Frequently repeated injections of the extract lead to the death of the animal preceded by weakness and general prostration.

A number of quite different factors seem to be involved in this thermic reaction. Further details and discussion will be presented later.

62 (1809)

## Relation of splenectomy to growth and appetite in the rat.

By **ARTHUR H. SMITH** and **LEAH ASCHAM.**

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

With a view of studying the alleged effect of splenectomy on appetite and growth,[1] experiments were carried out on white rats using the standard feeding technic of Osborne and Mendel. The growth was thus accurately measured and the qualitative as well as quantitative aspect of the food intake carefully controlled. Sixteen rats were splenectomized as nearly as possible at the age of 40 days. Of these, five were observed for 34 weeks, three for 43, and the remaining rats, which were the progeny of splenectomized parents, were observed for 23 weeks. In no case was there evidence of an increased appetite nor of variation from the normal growth rate.

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[1] Richet, *J. de Physiol. et de Pathol.*, 1912, xiv, 689; 1913, xv, 579. Prym, *Verhand. des Kongr. f. innere Med.*, 1911, xxviii, 398.

Erythrocyte count on five of the "second generation" splenectomized rats gave no indication of anemia following the operation.

### 63 (1810)

## Determination of optimum amount of antigen in complement fixation tests.

By **R. L. KAHN** and **S. R. JOHNSON**.

[*From Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.*]

A study of the quantitative relation between antigen and antibody in complement fixation, suggested a simple procedure for determining the optimum amount of antigen in these tests. Wassermann, protein and bacterial antigens were employed with their specific antisera. It was observed in the case of the Wassermann antigens (alcoholic, cholesterinized and Noguchi), that each one appears to possess an optimum concentration for binding complement with positive sera. This concentration could be determined only with weak positive sera, preferably those giving + and ++ reactions. The stronger positive sera do not seem to be markedly affected by the quantity of antigen employed.

The procedure consists in first determining the antigenic unit, or smallest quantity which gives complete fixation with some positive serum. A weekly positive serum is then pipetted into a series of 10 tubes, employing the same quantity used in the regular tests. The first tube then gets $\frac{1}{4}$ unit antigen; the second, $\frac{1}{2}$ unit; the third, 1 unit, etc.; the last tube getting about 10 units of this ingredient. After the fixation period, it will be observed, on adding sensitized cells, that certain tubes—not necessarily those containing the largest amount of antigen—will show the maximum amount of fixation. The number of antigenic units contained in these tubes being known, therefore, that number showing the maximum fixation, represents the optimum amount of antigen to be used in the daily tests. Necessarily this titration is to be repeated with 4 or 5 different sera.

In the case of the protein (edestin and phaseolin) and bacterial (*B. abortus* and *B. mallei*) antigen-antibody complexes, it was observed, after obtaining the antigenic unit, that increasing the number of units within the limitations of the complement fixation test, did not affect the strength of the reaction. One unit and as many as 8 units of antigen were found to give similar results. It would appear that the optimum amount of specific antigen for complement fixation tests is not the largest amount which may produce fixation, in view of the unnecessary increase in colloidal ingredients, but rather the smallest amount conducive to safety, as for example, 2 units.

### 64 (1811)

## The prevention and control of parathyroid tetany.

By ARNO B. LUCKHARDT and PHILIP J. ROSENBLOOM.

[*From the Hull Physiological Laboratory, The University of Chicago, Chicago, Ill.*]

If the signs and symptoms following parathyroidectomy are the result of an intoxication, as some investigators believe (Paton, Findlay, Watson, Burns, Sharpe, et alii), a vigorous diuresis if more or less continously maintained by means of the intravenous injections of physiological saline solutions might prevent the onset of tetany or rapidly lead to a disappearance of all symptoms of tetany if the tetany was first allowed to develop, providing the poison or poisons responsible for the condition were water-soluble and were excreted by the kidneys.

Dogs were accordingly injected intravenously two or three times daily with ordinary Ringer's solution following thyroparathyroidectomy. All injections were made with a Woodyatt pump delivering 42 c.c. per minute. The animals received 33 c.c. or more per kilo body weight at each injection. In some animals calcium-free Ringer's solution was injected from the start. In others, we changed from ordinary Ringer's solution to a calcium-free Ringer's solution to study the importance of the calcium ion in the Ringer's solution. The animals were fed a mixed diet consisting chiefly of meat.

Our chief results can be enumerated as follows:

1. By maintaining a brisk diuresis by means of intravenous injections of Ringer's solution it is possible to keep dogs alive indefinitely (at least two months) even when fed daily on a diet consisting chiefly of meat. The animals usually remain in a good state of nutrition. We have one animal which survived complete parathyroidectomy 51 days. In this animal we could induce symptoms of marked parathyroid tetany (hyperpnœa, anorexia, spasticity, tremors, and mild clonic convulsions) at will by stopping the injections and feeding the animal meat. Other animals have been kept alive for 14, 17, and 31 days. As far as we know they died because of an inability on our part to introduce enough Ringer's solution to maintain a vigorous diuresis.

2. Calcium-free Ringer's solution is quite as effective as ordinary Ringer's solution in prolonging the otherwise short life of a parathyroidectomized animal. After preventing the reappearance in one animal of severe tetany by the intravenous injections of Ringer's solution over a period of 26 days, we continued our treatment with calcium-free Ringer's solution for seven days with no change in condition of the animal. We next induced severe tetany by stopping all injections and feeding the animal a considerable amount of meat and cured the animal rapidly by forced injections of *calcium-free Ringer's solution.* The animal has been kept free from symptoms on calcium-free Ringer's solution up to the time of writing (eight days). It appears then that an active diuresis, however produced, with the elimination of toxic compounds (guanidine compounds, perhaps, as indicated by previous investigations) is more important than the administration of calcium compounds.

3. Marked appetite and consumption of food and a diuresis greater than one might expect on the basis of the known quantity of fluid injected seem to be consequences of the treatment. These and kindred phenomena are reserved for further investigation. A more detailed report of this work with a discussion of the possible importance of this method of treatment not only in parathyroid tetany but of allied conditions and diverse toxemias will appear shortly.

### 65 (1812)

# Testicular changes in acute alcoholism in man and their relationship to blastophthoria.

By CARL VERNON WELLER.

[*From the Department of Pathology, University of Michigan, Ann Arbor, Mich.*]

The blastophthoric effect of acute and chronic intoxications has been studied in the past chiefly by means of breeding experiments, in the course of which it has been shown that such agents as alcohol and lead can produce a definite blastophthoria without histologically demonstrable lesions in the germinal epithelium. By the same and other workers, it has been shown, however, that if the intoxication with these agents be increased, or if the subject be peculiarly susceptible, the germinal epithelium (male) may be made to exhibit degenerative changes, shown by atypical spermatogenesis or even by marked vacuolar degeneration and complete aspermatogenesis. It must be assumed as a working hypothesis that with any agent producing such demonstrable changes in the spermatogenetic process there is an earlier period in which spermatozoa showing less morphological deviation from the normal and capable of fertilizing are produced. Of great importance in this connection is the work of Widakowich[1] who has shown that the semen of syphilitics often contains increased numbers of atypical spermatozoa showing two, three or four heads; two, three or four tails, or combinations of such failures to divide. He notes also the occurrence of microcephalic and macrocephalic types.

In human material it is almost impossible to determine the relative effects of chronic and of acute alcoholism; so that, while the earlier literature specifies in almost all cases that the changes found are those of chronic alcoholism, the more acute degenerative changes and disturbances in spermatogenesis that have been described may be chiefly due to the acute exacerbations.

In investigation of this point five coroner's autopsies were selected having in common the facts that death occurred in, or immediately after a period of severe alcoholic intoxication and that the testes did not show interstitial or vascular fibroid changes such as might be expected if a severe intoxication of any sort had been long operative. The testes of these five cases all showed abnormal spermatogenesis. Vacuolar degeneration of the germinal epithelium, often showing a zonal distribution in the tubules; hyperchromatic spermatogonia, atypical division figures with hyperchromatic nuclei; retardation of spermatogenesis with relative increase in the number of spermatids and the formation of multinuclear forms attached to the wall or free in the tubular lumina, were noted in varying degrees in the different cases. These changes are not specific for alcohol but resemble those experimentally produced in laboratory animals by alcohol and lead, and those described as resulting from certain acute infections in man (typhoid fever, influenza and pneumonia). Whether, in the five cases here studied, the testicular changes were a direct or an indirect result of the acute alcoholism can not now be stated. A causal relationship of some sort seems evident from the constancy of the changes and from the supporting experimental evidence. The changes found are in excess of those which it is necessary to produce in the testis experimentally in order to demonstrate a blastophthoria by breeding experiments. It seems quite certain that in an earlier stage spermatozoa must have been produced still capable of fertilization, but incapable of producing normal offspring. The observation here recorded is therefore considered an additional contribution to the subject of human alcoholic blastophthoria.

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[1] Widakowich, *La Semana Médica*, 1920, No. 46, Buenos Aires.

### 66 (1813)

## A statistical study of the form and growth of a spore-bearing bacillus.

By **ARTHUR T. HENRICI.**

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

The rate of growth of *Bacillus megatherium* has been measured by direct counting of the cells, using a hæmocytometer; and the average length of the cells has been determined by measurements made at the same time. In agar cultures inoculated from a 12-hour agar culture (which has nearly reached the maximum of growth but has not yet formed spores) it was found that the cells began to increase in size during the lag phase and reached a maximum length, about six times that of the inoculated cells, shortly after the beginning of the maximum growth phase, then rapidly becoming shorter. During the period of increase in length, frequency curves showed a tendency towards bimodality, indicating that possibly a process of selection of rapidly growing cells may occur during the lag phase, as has been suggested by some investigators.

Two series of broth cultures inoculated from a 7-hour agar culture (during the period of maximum growth) showed no lag phase; nevertheless an increase in the size of the cells was observed beginning two hours after inoculation. The cells did not become so large as did those on agar, and the variation was not so great, bimodality being present in but one of the frequency curves. One series was inoculated with 10 times as many bacteria as the other, and the series receiving the lesser amount of inoculum showed a slightly greater increase in the size of the cells over a slightly longer period of time.

### 67 (1814)

## On the weight increments of premature infants as compared with those of fetuses of the same gestation age and those of full-term children.

By RICHARD E. SCAMMON.

[*From the Department of Anatomy, University of Minnesota, Minneapolis, Minn.*]

One method of approaching the problem of the effect of birth and the postnatal environment on the course of human growth is by the comparison of the rates of growth of premature infants with the growth rates of fetuses of the same gestation age and with those of full-term children. If the environmental factors are the all-important ones it might be expected a priori that the rate of growth of prematures would agree, in the main, with that of full-term children. If, on the other hand, the effects of the extrauterine environment do not seriously modify the course of growth established in prenatal life, it is to be expected that the curve of rate of growth in prematures will follow in general that of the fetus of the same gestation age.

The following study was based upon the weight records of 78 premature infants. In collecting the histories all cases were included which made any gain in weight in the first month after birth. These cases were divided in four groups according to their birth weight, and the rate of growth in the form of the monthly percentage increment in weight was determined separately for each case. The mean monthly increment of each groud for each month was then determined by averaging these individual percentages. The results obtained are shown in the table below. It will be noted: first, that the percentage increment in weight of prematures in the first postnatal month is lower than in the second month, and that following the second month the rate of increment gradually decreases; and, second, the percentage increments are in a general way inversely proportional to the birth weight.

AVERAGE MONTHLY PERCENTAGE INCREMENTS IN BODY-WEIGHT OF PREMATURE AND FULL-TERM CHILDREN IN INFANCY.

| | Group. | | | |
|---|---|---|---|---|
| | A | B | C | D |
| Range in birth-weight (grams) | 1,000<br>to<br>1,500 | 1,500<br>to<br>2,000 | 2,000<br>to<br>2,500 | ca. 2,750<br>to<br>ca. 4,200 |
| Approximate average birth weight (grams) | *1,300* | *1,720* | *2,300* | *3,380* |
| Total number of cases | 17 | 35 | 26 | * |
| Average percentage increment in: | | | | |
| First month | 22.8 | 16.8 | 13.8 | 21.2 |
| Second month | 45.1 | 31.6 | 26.7 | 19.4 |
| Third month | 24.5 | 20.4 | 15.5 | 14.6 |
| Fourth month | 21.1 | 17.2 | 13.5 | 11.4 |
| Fifth month | 16.2 | 13.6 | — | 8.9 |
| Sixth month | 14.4 | — | — | 5.8 |
| Seventh month | 11.6 | — | — | 5.9 |
| Eighth month | 7.4 | — | — | 3.9 |
| Ninth month | 5.1 | — | — | 4.0 |

* Average of ten large published series of observations.

The increments thus determined were next compared with those of fetuses of the same gestation age and with those of full-term newborn children.

The norm for fetal growth in weight was estimated from the following empirical formulæ: (1) $Y = 0.24\ X^{3.2} + 400$, where $Y$ is the body-weight in grams and $X$ is the body length in cm., and (2) $Y = 8.9\ (X - 1) - 0.27\ (X - 1)^2 - 6.5$, where $Y$ is the body length in cm. and $X$ is the age in fetal or lunar months. The norm for weight increment in the first nine postnatal months was determined by calculation from 10 large series of published averages on the increase in body weight in the first year. When the monthly weight increment rates of the premature infants are compared with these norms it is found that they fall much closer to the calculated rates of growth of fetuses of the same size and age than to those of newborn children. This is shown particularly well by group A of the smallest prematures which were approximately 7 fetal months old when born. The comparison is shown graphically in the chart below.

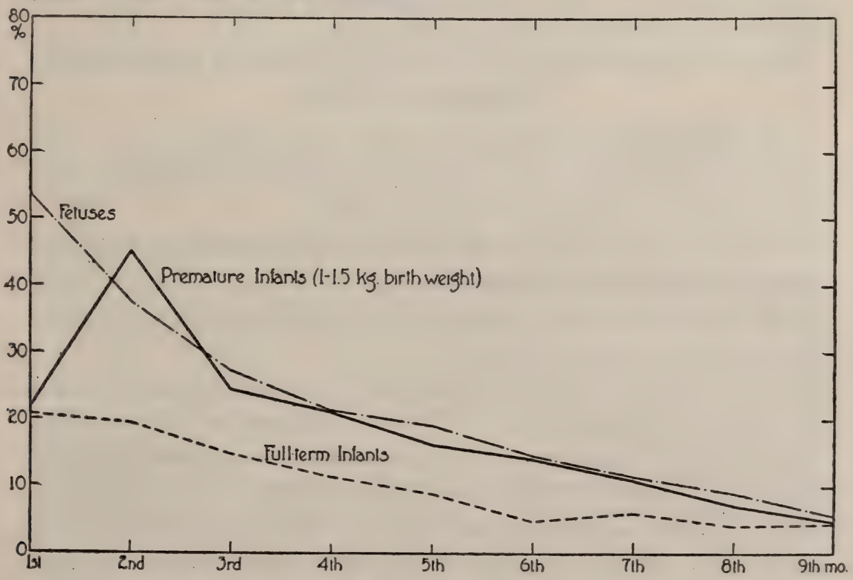

FIG. 1. A graph showing monthly percentage increment in weight of a group of premature infants ranging from 1.0 to 1.5 kg. in birth weight. The solid line represents the rate of increment of the premature children in the first 9 months after birth. The upper broken line represents the calculated rate of increment of the fetuses of the same size and age as the prematures. The lower broken line represents the rate of increment of full-term children.

These results indicate that premature children, after a short period of retarded growth incident to the adjustment to the extrauterine environment, tend to regain the fetal rate of growth and to follow this course of growth until some time in the latter part of the first year when the rates of fetal and postnatal growth approximate one another. In other words the growth tendency of prematures is in general that of fetuses of the same size and age rather than that of full-term children. These results are in agreement with those of Hammett[1] on growth capacity and body weight in the first two weeks of postnatal life, and with certain findings of Cammerer,[2] but seem to be in opposition to some of the conclusions of Schwarz and Kohn[3] and of Ylppö.[4] That this opposition is apparent rather than real will be shown in a later communication.

### 68 (1815)

## An undetermined principle obtained from poison ivy.

By E. D. BROWN.

[*From the Department of Pharmacology, University of Minnesota, Minneapolis, Minn.*]

An undetermined principle has been obtained from poison ivy which so far as I have been able to find differs in its behavior from that of any substance previously described.

It came down as a precipitate after long standing of a filtrate after precipitating with lead acetate.

No work has as yet been done with the substance except to determine a few of its properties.

It is non-irritant when applied to the skin, neutral to litmus and is bitter to the taste. It has a melting point of 190° when heated slowly, insoluble in cold water and fairly soluble on boiling, imparting a lemon yellow color to the solution. It is soluble in ammonia water, acetic acid and hot alcohol. Insoluble in alcohol in the cold, ether, chloroform, petroleum ether and acetone. It gives striking color reactions with $H_2SO_4$, $HNO_3$, KOH, CaOH and other reagents.

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[1] Hammett, F. S., *Amer. Jour. Physiol.*, 1919, xlv, 396.

[2] Cammerer, W., *Jahrb. f. Kinderheilk.*, 1900, liii, 381.

[3] Schwarz and Kohn, J. L., *Amer. Jour. Dis. Children*, 1921, 296.

[4] Ylppö, *Zeitschr. f. Kinderheilk.*, 1919, xxiv, 179.

A solution to which a few drops of silver nitrate are added gives a pink color changing quickly to a dull green.

Iodine as Lugol's solution gives a pink color which quickly fades.

With ferric chloride a very dark blue or black changing to brown.

On the addition of Fehling's solution it turns green and on boiling a slight reduction occurs.

It does not reduce Fehling's solution in the cold on long standing even after it had been previously boiled with acid and again rendered neutral. This leads to doubt as to its being a glucoside.

With Millon's reagent it turns a port-wine color rapidly becoming darker which suggest the possibility of its belonging in the group of the phenols.

## 69 (1816)

## The effect of heat on the calcium salts and rennet coagulability of cow's milk.*

By LEROY S. PALMER.

[*From the Section of Dairy Chemistry, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

When milk is boiled a precipitation of a portion of the calcium phosphates occurs, the amount of fixation being proportional in general to the amount and duration of heat applied. Söldner[1] first called attention to this fact and his observations have been confirmed by numerous investigators, among whom may be mentioned Boekhout and de Vries,[2] Purvis, Brehaut and McHattie,[3] and Grosser.[4]

It has been commonly believed, also, that some fixation of the calcium phosphates takes place during the holding process of pasteurization. The fact that pasteurization of milk retards the coagulability of the casein by rennet and the fact that this property can be restored by the addition of calcium chloride to the milk have been presented in support of the view that heat changes some of the soluble calcium salts to an insoluble form. The experimental evidence for such a change is, however, contradictory. Solomin[1] noted that a little phosphorus falls out of milk when the temperature is raised to 80° C. Diffloth[2] found a decrease of 26 per cent. in the soluble phosphates when milk was held at 60° C. for 30 minutes. Rupp,[3] however, approached the problem by filtering raw and pasteurized milk through a clay filter and analyzing the filtrate for calcium and phosphorus. He found no change in the calcium and phosphorus after holding the milk for 30 minutes at 68.3° C. Milroy[4] held the fresh milk at a temperature just below the boiling point for one hour and, after filtering through an ordinary filter, noted a decline in calcium. He explained this result on the basis of a transformation of dicalcium phosphate into basic calcium phosphate. Grosser made similar studies on samples of milk which had been boiled for 5, 10 and 15 minutes, respectively, and noted a negligible loss in phosphorus in the filtrate, but a slight loss in calcium.

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* Published with the approval of the Director as Paper No. 288, Journal Series, Minnesota Agricultural Experiment Station.

[1] Söldner, F., *Landw. Versuchs.*, 1888, xxxv, 351.

[2] Boekhout, F. W. J., and de Vries, J. J. O., *Landw. Versuchs.*, 1901, lv, 221.

[3] Purvis, J. E., Brehaut, A. H., and McHattie, A. C. N., *Roy. Sanit. Inst. Journ. Trans.*, 1912, xxxiii, 154.

[4] Grosser, Paul, *Biochem. Zeitschr.*, 1913, xlviii, 427.

Daniels and Loughlin[5] have recently obtained qualitative evidence that calcium phosphates are thrown down when milk is pasteurized by the holding process, in that they have noted a nutritional calcium and phosphorus deficiency of such milk which could be prevented by feeding the washings from the walls of the vessel in which the milk was pasteurized or by addition of calcium phosphate to the rations of the animals (rats) in the experiments.

The explanation commonly held for the effect of heat on the calcium phosphates of milk originated with Söldner who believed that the calcium of milk is present as mono- and dicalcium phosphates which, on heating, pass to tricalcium phosphate and are thus precipitated. This explanation has apparently never been submitted to critical examination. Monocalcium phosphate is readily soluble in water and its solutions decompose on boiling giving rise to dicalcium phosphate whose solubility is so low that a heavy precipitation of phosphates occurs. The solubility of dicalcium phosphate is, however, only 0.135 to 0.561 part per 1000 of water, depending on the saturation of the water with $CO_2$. The more highly concentrated solution naturally gives up some of its calcium phosphate on heating, due to the loss of $CO_2$. The solution in pure water also clouds up on boiling.

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[1] Solomin, P., *Arch. f. Hyg.*, 1897, xxviii, 43.

[2] Diffloth, Paul, *Bull. d. Sci. Pharm.*, 1904, x, 273; *Zeit. Nahr. Genussm.*, 1906, xi, 455.

[3] Rupp, Philip, U. S. Dept. of Agr., Bureau of Animal Ind. Bull., 1913, clxvi, 1–16.

[4] Milroy, T. H., *Biochem. Jr.*, 1915, ix, 221.

[5] Daniels, A. L. and Loughlin, R. J., *Biol. Chem.*, 1920, xliv, 381.

The facts just cited seem to support, in general, Söldner's theory. However, the experimental results of Grosser[4] and Rupp,[7] cited above, show that there is actually little if any decrease in the calcium phosphates dissolved in milk when the milk is held at pasteurization temperatures or boiled for some minutes. At the same time there is abundant evidence, as indicated, that heat does precipitate calcium phosphates from milk. How are these divergencies in results to be explained?

It occurred to the writer that a simple explanation of these divergencies is afforded by the experimental evidence brought forth by Van Slyke and Bosworth[1] that the calcium phosphate of cow's milk is wholly in the form of dicalcium phosphate, amounting to about 1.75 parts per 1000, on the average. These figures are greatly in excess of the maximum solubility of dicalcium phosphate, even in water saturated with $CO_2$. These investigators found, moreover, that the dicalcium phosphate of cow's milk was retained on the Pasteur-Chamberland filter when milk is filtered through this medium. The natural conclusion to be drawn from these results is that the calcium phosphate of cow's milk, which appears to be wholly in the form of $CaHPO_4$, is present in colloidal solution, and that the aggregates of particles are sufficiently large that they do not pass through the Pasteur-Chamberland filter, or even through the Bechloidt filter used by Grosser, or the clay filter used by Rupp. This conclusion coincides with the results of Grosser and Rupp who obviously were not dealing with the colloidal matter of milk in their analyses of filtrates from the clay filters which they used. The experiments which have shown a gross decline in calcium phosphates or in which the precipitated phosphates have been seen, when milk is heated, are to be explained, therefore, solely by the effects of heat on colloidal solutions of dicalcium phosphate. As a matter of fact the loss of calcium and phosphorus from milk on boiling observed by Söldner showed a ratio of one molecule of calcium to one of phosphorus such as exists in dicalcium phosphate.

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[1] Van Slyke, L. L., and Bosworth, A. W., *N. Y. Agr. Exp. Sta. Tech. Bull.*, 1914, xxxix, 1–17.

## EXPERIMENTAL.

In order to determine what the effect of heat is on colloidal $CaHPO_4$ solutions, such a solution was prepared by grinding $CaHPO_4$, which had been washed free from electrolytes, to an impalpable powder in a porcelain ball mill. This powder was then ground further in the mill in the presence of a 0.6 per cent. gelatin solution. After settling, the supernatant fluid presented a very satisfactory colloidal suspension of $CaHPO_4$. It was distinctly milky and showed the usual Tyndall effects in a striking manner. The concentration of $CaHPO_4$ was not, however, as high as had been expected, the solution being found to contain only 0.542 gram per 1000. Possibly a higher concentration would have been obtained if a stronger solution of gelatin had been employed or a better colloid stabilizer used. Gelatin was chosen, however, because it is not coagulated by heat.

The effect of heat on this colloidal solution of $CaHPO_4$ was determined qualitatively only by heating a portion of it in a water bath at 63° C. for 30 minutes. A heavy precipitation of $CaHPO_4$ resulted and the filtrate showed much less evidence of a colooidal suspension.

This simple experiment shows rather conclusively that it is not necessary to assume any transformation in the composition of the calcium phosphates of milk during heating to account for the partial fixation of these salts. The phenomenon is readily accounted for by the effect of heat on a colloidal solution of $CaHPO_4$ which renders such a solution much less stable and causes the aggregates to pass, in part at least, to the crystalloid form.

The results secured in this experiment have a bearing, also, on the alleged effect of heat on the calcium salts of milk as affecting the coagulability of milk by rennet. It seems evident that the only calcium salts affected by heat are colloidal calcium salts and the question is therefore raised as to the possibility of the colloidal $CaHPO_4$ of milk playing a part in the rennet coagulability.

In order to determine whether this is true or not, two 200 c.c. portions of fresh whole milk were dialyzed in collodion bags against running distilled water for 48 hours, using 1 per cent. toluene as preservative. When rennet was added to this milk there was no coagulation even after several hours. One drop of 4 molar $CaCl_2$ solution added to 100 c.c. of the rennet treated milk caused instant coagulation. The same result followed the addition of 2 or 3 drops of dilute HCl solution. The addition of 10 c.c. of the colloidal gelatin solution of $CaHPO_4$ to 100 c.c. of the rennet treatment milk was, however, without any effect.

It is apparent that the colloidal $CaHPO_4$ of the milk does not play any part in the rennet coagulation. The indications are, also, that the effects of heat on rennet coagulation which can apparently be overcome by the addition of soluble calcium salts are not to be explained on the grounds of an effect of the heat on the calcium salts of the milk but rather on the grounds of an effect of heat on the casein itself. Just what this may be is not definitely clear, as yet. The author has this problem under investigation and hopes to be able to present definite data on it at a later date. It will be sufficient to point out at this time that the explanation of this phenomenon involves the fact that rennet coagulation is unquestionably both a chemical and a colloidal reaction. The calcium caseinate of milk is in colloidal solution. Rennet appears to hydrolyze the calcium caseinate into two molecules of calcium paracaseinate. The clotting of the calcium paracaseinate is a secondary phenomenon which is a gellation, perhaps of the nature of a crystallization of colloid—in this case a hydrophyllic colloid in a state of hydration. The conditions which govern what is regarded as a normal clotting of the calcium paracaseinate are evidently disturbed by the application of heat to the colloidal calcium caseinate of the milk. Zoller[1] has recently shown how the properties of the casein of milk are affected by heat so as to have a marked influence on the precipitation of casein by acids and on the hydrophyllic properties of the casein thus precipitated. Apparently the addition of soluble calcium salts to milk helps to restore the conditions existing in raw milk which govern the normal rennet clot. All the interrelations of calcium and rennet coagulation have obviously not been determined. A study of these relations is at present in progress in this laboratory.

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[1] Zoller, H. F., *J. Ind. Eng. Chem.*, 1921, xiii, 510.

## SUMMARY.

It is shown that the partial fixation of the calcium salts of milk by pasteurization or boiling is readily explained simply on the grounds of the effect of heat on colloidal solutions of $CaHPO_4$, the calcium phosphate natural to cow's milk.

It is shown further that the effect of heat in retarding the rennet coagulability of milk is not related directly to the loss of colloidal $CaHPO_4$ because the addition of colloidal $CaHPO_4$ to dialyzed milk does not restore its coagulation by rennet, while the addition of $CaCl_2$ or HCl does restore this property.

The phenomenon of rennet coagulation is discussed briefly from the standpoint of the chemical and physico-chemical reactions involved, and also from the standpoint of the possible bearing which the addition of calcium salts to heated milk has on this phenomenon.

### 70 (1817)

## The velocity of development of the demarcation current in the frog's sartorius.

By GEORGE EDMESTON FAHR.

[*From the Department of Medicine, University of Minnesota, Minneapolis, Minn.*]

Urano and Fahr have experimentally established the fact that the potassium ion is almost exclusively the only cation within the muscle cell of the frog's sartorius. Overton has shown that the demarcation current of the frog's sartorius may be inhibited or have its sign reversed by replacing the lymph fluid surrounding the muscle cell by a fluid containing K ions in place of the normally

Na ions. This action is reversible. The author has repeated Overton's experiments and in addition to confirming them found that the relation of K in the muscle cell bathing fluid to the potential developed by injury of the muscle is a quantitative one and also that it is possible to get not only a change of direction for the demarcation current by replacing Na in the cell bathing fluid by sufficient K but also to get a pseudo action current under these circumstances. This pseudo action current only travels a short distance along the muscle, is of slow rate of progression and is accompanied by a small, slow contraction.

From the above experiments it was concluded that a displacement of K ions across a semi-permeable cell boundary was responsible for the demarcation current of muscle. At the moment of cutting or injuring a muscle cell there is opportunity for ion equilibrium at the cut surface, whereas there is ion and thus electric strain at the uncut surface. If this hypothesis is true the velocity with which the demarcation current develops to its maximum intensity is of the order of 1/100,000 of seconds because of the speed of ions and the distance to be travelled by them. Garten has cut the surface of the frog's sartorius and determined the speed with which the demarcation current rises to its maximum value by means of the capillary electrometer. He believes that his experiments show that more than 1/1,000 of second is necessary for the demarcation current to develop.

The analysis of Garten's capillary electrometer curves is based on the formula $D(ds/dt) + Ks = Ci$. This formula neglects the mass factor because usually the mass is so small in relation to the friction that it may be neglected.

$$M\frac{d^2s}{dt^2} + D\frac{ds}{dt} + Ks = Ci$$

is the equation which accurately describes the forces acting on the capillary when a potential difference is applied to it.

Curves analyzed according to the first formula do not give an accurate picture of the development of potential difference during the first 1/1,000 of a second. Therefore we do not believe that Garten has proved that it takes more than 1/1,000 of a second to fully establish the current of injury of a frog's muscle.

In the year 1913 the author attempted to solve this question in the physiological laboratory of the University of Giessen under Professor Garten.

To establish the current of injury a frog's sartorius muscle was cut by a rifle bullet. This bullet cut a copper wire just before entering the muscle substance. The copper wire was shunted across a string galvanometer through which a constant current was passing. This galvonometer immediately responded with an excursion which was used as time marker. After passing the muscle the bullet cut another copper wire shunted across a second galvanometer. This galvanometer was connected to one electrode at the end of the muscle farthest from the injured portion. The other electrode lay beneath the injured portion of the muscle. It was possible to cut all but a few fibers of the muscle by means of the bullet and keep the muscle firmly attached to the electrodes. The moment the rifle trigger was pulled a hymographion carrying film was shot at the speed of 4,000 mms. per second. The galvanometer excursions were recorded on this. It was possible to get uniform velocity over a large portion of the film. A time marker recorded the time and ordinates were established as well as abscissæ by mechanical devises. After recording a current of injury the two copper wires were replaced and a bullet shot through them as before. In this experiment a constant current equal to the previously measured demarcation current was thrown through the galvanometer at the moment the second copper wire was cut. We thus had a picture of the excursion of the string under the influence of a constant current to compare with our demarcation current. It was possible to measure time with an error of less than 1/10,000 of a second because of the speed of the film and the uniformity of the velocity of the hymographion. Apparently all the demarcation current excursions of the string reproduced the constant current excursions within the limits of errors of the method. That is the curves covered one another so closely that one could infer that the demarcation current is established with its maximum value within 1/10,000 of a second after injury. The strongest objection to the ion theory of the demarcation current is therefore removed.

71 (1818)

## The pharmacological action of some ethers and esters of saligenin.[1]

By **ARTHUR D. HIRSCHFELDER** and **HERMAN H. JENSEN.**

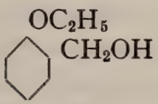
[*From the Department of Pharmacology, University of Minnesota, Minneapolis, Minn.*]

Benzyl alcohol saligenin and other aromatic alcohols have been shown to possess local anesthetic and antispasmodic action (Macht; Hirschfelder; Hjort). Hirschfelder and Quigley have also demonstrated that the local anesthetic action of benzyl alcohol and its derivatives is diminished when one of the inactive hydrogens (*i.e.*, in the $CH_2$ of the $CH_2OH$ carbinol group) is substituted by another radical; or, in other words, that the secondary aromatic alcohols are not as good local anesthetics as the primary, and that substitutions for both the $CH_2$ hydrogens (tertiary alcohols) causes complete loss of local anesthetic action.

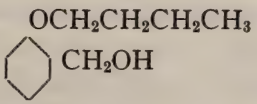
Although Hirschfelder, Lundholm and Norrgard had demonstrated that methyl and ethyl substitutions on the phenolic hydroxyl of saligenin rendered the substances more irritating than saligenin, a more extensive study of this type of substitution products was desirable, particularly on account of the fact that they furnished some alcohols homologous with acetyl-salicylic acid.

The substances studied may be devided into three groups:

I. Ethers of saligenin with substitution on the phenolic hydroxyl: *i.e.*, the ethyl, *n*-butyl, iso-amyl and benzyl ethers.



I.
Ethyl saligenin.



II.
*N*-Butyl saligenin.

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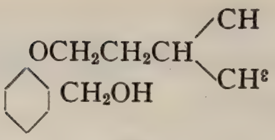
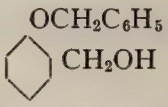
[1] The substances used in this research were prepared by Merrill C. Hart as by-products of an investigation of the phenolic alcohols and their derivatives as antiseptics and for the chemotherapy of the venereal diseases, with the aid of funds furnished by the United States Interdepartmental Social Hygiene Board. Their preparation has already been described elsewhere (M. C. Hart and A. D. Hirschfelder, *Jour. Am. Chem. Soc.*, 1921, July).

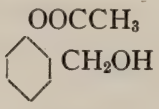


III.
Iso-amyl saligenin.

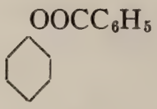


IV.
Benzyl saligenin.

II. Esters of saligenin with substitution on the phenolic hydroxyl (acetyl and monobenzoyl saligenin).

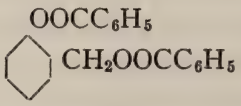


V.
Acetyl saligenin.



VI.
Benzoyl saligenin.

III. An ester with substitution on both hydroxyls (dibenzoyl saligenin).



VI.
Dibenzoyl saligenin.

All of these esters and ethers except the dibenzoyl compound are oily liquids, and the latter is a solid; and all are practically insoluble in water but soluble in the usual organic solvents and in olive oil. Their pharmacological properties were therefore studied by dissolving the substances in olive oil and emulsifying this with acacia by the Continental method.

Toxicity tests, made by determining the dose which was lethal for frogs in twenty-four hours, gave the following results: For the ethers, ethyl saligenin 0.5 mg. per gram frog, *n*-butyl 0.25 mg., iso-amyl 0.12, benzyl 0.36 to 4 mg.; for the esters, acetyl saligenin 0.9 mg., benzoyl 1.0 mg., dibenzoyl 2.0 to 3.0 mg. All of these compounds are therefore more toxic than saligenin itself.

The local anesthetic action was tested by dipping the frog's foot into the emulsion and then into one per cent. sulphuric acid. Two per cent. emulsions were used. Anesthetic action set in from two to five minutes after exposure to the drug and lasted from ten to twenty-five minutes, except in the case of the normal butyl ether which was more prolonged and lasted from one to two hours, thus being more prolonged than that of saligenin. With the dibenzoyl ester no anesthesia whatever was obtained, even after thirty minutes' exposure to a five per cent. emulsion. This corresponds to the results which have been obtained with benzyl benzoate and other benzyl esters by Macht and others.

All these substances are, however, very irritating. On the tongues of human beings they give rise to a bitter taste and an intense burning sensation which is most marked with the normal butyl, and least marked with the dibenzoyl ester.

Upon the contractions of excised segments of rabbits' duodenum in 400 mils of aërated Ringer-Langendorff solution at 38–39 the addition of 2.5 mils of ethyl saligenin decreased the amplitude, slowed the rate, and finally caused complete inhibition. 1.5 mils of *n*-butyl, 0.5 mil of iso-amyl, 1.0 mil of benzoyl ester, and 10.0 mils of the dibenzoyl ester produced the same effects; but only 0.8 mil of the benzyl ether was required to produce complete inhibition, accompanied by a very marked lowering of tone. This lowering of tone was also striking with the iso-amyl but not with the other ethers. In the case of the acetyl ester there was at first an increased amplitude (probably due to acetic acid from hydrolysis) with slowing of the rate, gradually followed by inhibition.

When injected intravenously into starved rabbits, anaesthetized with ether, no visible effect upon the contractions of the exposed small intestine could be observed through a glass window in the abdominal wall after the ethyl and the iso-amyl ethers, but the *n*-butyl, the benzyl, the benzoyl and the dibenzoyl compounds all caused a definite inhibition of peristalsis and a well-marked dilatation of the intestine. Contrary to the findings of Mason and Piek, and in accord with the experiments of Macht, we also observed this inhibition after the injection of benzyl benzoate. When applied locally to the rabbit's intestine, all our compounds, with one exception, caused inhibition and dilatation. The acetic ester, however, on intravenous injection augmented the peristalsis, and when applied locally produced spastic contractions.

All the emulsions, on intravenous injection, caused a fall of blood pressure, which varied from a sudden transitory fall in the case of the ethyl, *n*-butyl and iso-amyl ethers to a more gradual and more prolonged fall after the acetyl, benzoyl and dibenzoyl esters. The benzyl ether caused a sudden and more prolonged fall, which lasted four to five minutes. Control emulsions, injected at the same slow rate, gave no effect whatever. Perfusion of the frog's circulatory system gave a marked vaso constrictor effect, in striking contrast to the vasodilation obtained with emulsions of saligenin and benzyl benzoate. This constriction is probably due to irritation of the arterial walls.

# SCIENTIFIC PROCEEDINGS.

## ABSTRACTS OF COMMUNICATIONS.

### One hundred twentieth meeting.

*College of Physicians and Surgeons, January 18, 1922.*
*President Wallace in the chair.*

72 (1819)

### Is there more than one kind of rickets?[1]

By **E. A. PARK, P. G. SHIPLEY, E. V. MCCOLLUM** and **NINA SIMMONDS.**

[*From the Department of Pediatrics, Yale University, New Haven, Conn., the Department of Pediatrics, Johns Hopkins University, and the Department of Chemical Hygiene, Johns Hopkins University, Baltimore, Md.*]

#### I. CLINICAL OBSERVATIONS SUGGESTING THE EXISTENCE OF MORE THAN ONE KIND OF RICKETS.

For at least two years our attention has been attracted to the possibility that there might be more than one kind of rickets. We were led to think of this possibility as the result of the consideration of certain peculiar manifestations of the disease and associations with other diseases. The facts are as follows: Rickets occurs with great frequency in premature children, even when breast fed. It seems to affect the head more than the extremities or ribs. In a group of cases of rickets the disease shows an especial tendency to involve the shafts of the long bones declaring itself clinically by the occurrence of multiple fractures from trivial causes. There is a curious association between rickets and certain forms of secondary anemia (the so-called alimentary anemias and the anemias of the von Jaksch type). The rachitic involvement of the head in the children suffering from the combined conditions appears to be out of all proportion to the involvement of the extremities. Rickets is associated with the most severe forms of chronic interstitial nephritis or developmental defects of the kidney in which there is the most extreme degree of disturbance in renal function. Curiously, tetany sometimes occurs with rickets and sometimes not. Since the conception that there might be more than one kind of rickets was supported solely by clinical observations of at most a suggestive character, we did not feel at liberty to express it as a definite hypothesis. Recently, however, our experiments have yielded results which indicate that there may be two distinct forms of the disease.

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[1] Abstract presented at the one hundred nineteenth meeting of the Society for Experimental Biology and Medicine.

## II. THE EXPERIMENTAL EVIDENCE.

In previous publications[1] we have described certain defective diets which, when fed to the young rat, produced marked disturbances in the growth and calcification of the skeleton. The diets in question were all insufficiently supplied with a factor or factors present in cod liver oil. They differed from each other considerably, however, in the composition of their mineral fraction, chiefly, however, as regards the calcium and phosphorus. They may be divided into two groups according to the relative amounts of those two elements present. In one group, the phosphorus was at a low level but the calcium at or above the optimal level; in the other group, on the other hand, the calcium was at an extremely low level but the phosphorus was not far from the optimal.

When the diets of the first group (the phosphorus being deficient and the calcium-phosphate ratio high) were fed to young rats living under ordinary laboratory conditions (room light), there developed a diseased condition of the skeleton which was identical in all essential particulars with that seen in the rickets of human beings. The costo-chondral junctions were greatly enlarged; in some animals the thoracic wall was sunken at the sites of the costo-chondral junctions and the shafts of the ribs were fractured. The long bones of the extremities were enlarged at the ends; they could be cut and broken easily. Between shaft and cartilage lay a yellowish zone two to three mm. deep, the rachitic metaphysis. The proliferative cartilage extended in irregular prolongations toward the shaft. Calcium deposition in the cartilage was entirely lacking or extremely defective. The intermediate zone between cartilage and shaft presented the picture typical of the metaphysis in the bones of rachitic children. It was composed of cartilage in all stages of metaplasia or degeneration into a material indistinguishable from the osteoid, trabeculæ consisting of osteoid, blood vessels bordered by marrow elements, scattered, irregular deposits of calcified material incased in osteoid, and connective tissue. All were intermingled in a disorderly manner. The trabeculæ of the shaft were surrounded by broad investments of osteoid.

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[1] McCollum, E. V., Simmonds, Nina, Parsons, H. T., Shipley, P. G., and Park, E. A. *Jour. Biol. Chem.*, 1921, xlv, 333.

Shipley, P. G., Park, E. A., McCollum, E. V., and Simmonds, Nina. *The Johns Hopkins Bulletin*, 1921, xxxii, 160.

McCollum, E. V., Simmonds, Nina, Shipley, P. G., and Park, E. A. *Amer. Jour. of Hygiene*, 1921, i, 492.

McCollum, E. V., Simmonds, Nina, Shipley, P. G., and Park, E. A. *Jour. Biol. Chem.*, 1921, xlvii, 507.

When the diets of the second group (the calcium being deficient, the phosphorus at a level not far from the optimal and the calcium-phosphate ratio low) were fed to rats kept under ordinary laboratory conditions (room light), there developed a diseased condition of the skeleton which also bore marked resemblances to the lesions found in the rickets of human beings. The gross deformities caused by the second group of diets were as great or greater than those caused by the first group and corresponded exactly to the deformities found in rachitic children. The thorax was even more deformed than in the rats fed the diets of the first group; it was flattened from side to side and marked at the sides by deep grooves following the costo-chondral junctions; the angular deformities produced by the costal cartilages and the shafts projected into its interior; the costo-chondral junctions were enlarged and greatly distorted; fractures in the shafts of the ribs were especially numerous. The lower ends of radius and ulna were enlarged as were also the ends of all the long bones of the extremities. The bones were extremely soft and weak. Between the cartilage and the shaft was a white intermediate zone one to three mm. deep. Microscopic examination showed that the cartilage was entirely free from calcium or nearly free from it and was invaded in an irregular manner by the vascular elements of the shaft. In consequence the cartilage extended toward the shaft in irregular prolongations. The cells of the cartilage in proximity to the shaft showed evidences of degeneration and metaplasia. The intermediate zone was composed of cartilage in a more or less degenerated state, osteoid trabeculæ, blood vessels surrounded by marrow elements, a few deposits of calcium for the most part situated near the periphery and connective tissue. The trabeculæ of the shaft were bordered by rather broad zones of osteoid. A loosely arranged fibrous tissue invested many of the trabeculæ. In those places in which it filled in the spaces between them, it gave rise to histological pictures which closely resembled those presented by the fibrous marrow in the rickets of human beings.

The pathological condition induced in the bones by the diets of the second group did not, however, exactly correspond at all points to that usually found in the human subjects of the disease. The cartilage was invaded and its columnar arrangement was disrupted to a less extent than is commonly the case in the rickets of human beings. The metaphysis was composed in larger part by osteoid trabeculæ. Though these osteoid trabeculæ were free from calcium deposition, they, nevertheless, retained a certain semblance of orderly arrangement. The osteoid zones about the trabeculæ were not so broad as in the rats on the diets of the first group, though they were quite as broad as the osteoid borders in the bones of rachitic children. Cells evidently derived from the fixed tissues with large basophilic granulations were numerous in the immediate vicinity of the trabeculæ. Resorptive activity was exceedingly marked. In the fundamental respects, however, in particular, degeneration and metaplastic changes in the cartilage, defective calcification of the cartilage and trabeculæ and the consequent osteoid production, the irregular invasion of the cartilage and the production of a rachitic intermediary zone, the pathological conditions produced by the faulty diets of the second group corresponded to the rickets of human beings. While one of the diets in question, diet 2638, did not give constant results, in some animals it produced a pathological condition corresponding to that found in human rickets even in its minor details.

## III. DISCUSSION.

Our experiments make it clear that in the rat, when deprived of certain active light rays and an unidentified factor contained in cod liver oil, a pathological condition corresponding in all fundamental respects to the rickets of the human being can be produced through the diet in two ways; it can be produced (1) by diminishing the phosphorus in the diet and supplying calcium in excess of the optimal or at the optimal concentration, or (2) by reducing the calcium but maintaining the phosphorus at a concentration somewhere near the optimal. In the former case the calcium-phosphate ratio in the diet is large, in the latter case it is small. We have not the slightest doubt that in the human being similarly deprived of active light and the unidentified factor it would be possible to produce true rickets through a manipulation of the calcium and phosphorus of the diet in the two ways mentioned. As the result of our experiments we are led to believe, therefore, that there are two main forms of rickets, one characterized by a normal or nearly normal blood calcium and a low phosphorus, the calcium-phosphate ratio being high, the other by a normal or nearly normal blood phosphorus but a low calcium, the calcium-phosphate ratio being low. Between the two forms there are probably innumerable intermediary forms marked by calcium-phosphate ratios which are less effective in preventing calcium-phosphate deposition. Diets with these intermediary ratios manifest themselves pathologically as rickets in various stages of healing or of healing and relapse or as slight disturbances of calcification of the skeleton which have more or less remote resemblance to rickets.

At one time we were under the impression that tetany sharply marked off one form from the other, *i.e.*, that the two main divisions of rickets were ordinary rickets and the rickets of tetany. The investigations of Howland and Kramer[1] have shown, however, that tetany *in a latent* form, at least, may be associated with the low phosphorus form of the disease. Though tetany may occur with the low phosphorus form, however, it is probably regularly associated with the low calcium form and may be regarded as a symptom of the latter. The low calcium form of rickets is, generally speaking, the rickets of tetany. Tetany may of course occur independently of rickets. If tetany persists long enough, however, evidence of defective calcification of the skeleton would almost certainly develop.

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[1] Howland, John, and Kramer, Benjamin. *Amer. Jour. of Dis. of Child.*, 1921, xxii, 105.

On theoretical grounds entirely, we think it possible that the rickets which develops in the youthful subjects of the severe functional derangements of the kidney may belong to the low calcium form of the disease; in other words, that the rickets in these patients may be truly endogenous in origin depending primarily on the inability of the kidney to excrete phosphorus. If this view proves to be correct "renal dwarfism" [1] is in reality renal rickets and ought so to be called and regarded.

### 73 (1820)

## Variations in aliquot fractions of gastric contents.

### By NICHOLAS KOPELOFF.

*[From the Department of Bacteriology, Psychiatric Institute, Ward's Island, New York City.]*

Aliquot fractions obtained by the Rehfuss method of fractional gastric analysis do not accurately represent the total gastric contents, as indicated by the results of the following experiments on subjects showing no clinical evidence of gastric disease:

1. Instead of the usual periodic aspiration, the total gastric contents were removed after three quarters of an hour by withdrawing 10 c.c. fractions in rapid succession. A wide variation was found in the acidity of these fractions, indicating that the total gastric contents are not a homogeneous mixture and that a single 10 c.c. sample is not a valid aliquot. This was more noticeable in subjects having a high rather than low gastric acidity.

2. (*a*) By inserting *three* Rehfuss tubes in one individual and aspirating the fractions simultaneously at fifteen minute intervals, it was found that there was considerable variation of acidity in different parts of the stomach at the same moment. X-ray pictures established the relative position of the tubes.

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[1] Barber, Hugh. *Quart. Jour. of Med.*, 1921, xiv, No. 55.

(*b*) From these data it is shown that widely divergent curves of acidity may be plotted which depend entirely upon the experimental error of the method and not upon the subject's gastric condition.

(*c*) The inadequacy of the titration method and the importance of hydrogen ions and buffer salts in measuring gastric acidity is indicated.

3. A subject who could regurgitate his total gastric contents at will, was given the test meal on different days and the total contents were regurgitated at different intervals from the time of ingestion to the test meal. The curve of acidity plotted in this manner differed radically from the curve obtained in the usual manner. Furthermore, discrepancies were noted between the acidity of an aliquot removed immediately prior to regurgitation and the acidity of the total gastric contents.

Results obtained by the Rehfuss method may be more validly interpreted if: (*a*) the analysis is repeated until a satisfactory agreement in curves is obtained; (*b*) the tube is kept at a constant level; (*c*) aliquot fractions are large; (*d*) little saliva is swallowed; (*e*) acidity is measured in terms of hydrogen ions and buffer salts.

### 74 (1821)

## The effect of cooking upon the antiscorbutic vitamin in cabbage.

By **WALTER H. EDDY, E. SHELOW** and **R. A. PEASE.**

[*From the Department of Physiological Chemistry and the Department of Foods and Cookery, Teachers College, Columbia University, New York City.*]

The present report is one of a series of studies undertaken to determine the effect of the new cooking implement known as the pressure cooker upon the vitamin content of such foods as are adapted to preparation in that device. The cooker also affords in its manipulation an opportunity to throw light on the response of vitamins to certain combinations of destructive influences that are absent or different from those met in older methods of cookery. The present study is confined to a comparison of the effects of pressure versus open kettle cooking on the vitamin C content of cabbage.

In open kettle cooking the routine involves immersing a given wt. of cabbage in a kettle containing one cup of boiling water and continuing the boiling until the cabbage is in a condition suitable for the table. This required from 45 to 90 minutes according to the weight of cabbage used. In bringing the cabbage to the same condition with the pressure cooker the following procedure was necessary: One cup of water was placed in the cooker and brought to boiling. Cabbage was then added, the cover clamped on and the cooker placed over the flame. The valve was left open until dry steam issued, then closed and heating continued until a pressure of 15 lbs. was reached and a temperature of 121° C. From the time of adding the cabbage to this point required 3–5 minutes. The flame was then regulated to maintain this pressure and temperature for thirteen minutes. At the end of that time the cooker was removed from the flame and allowed to cool until the pressure had dropped to 5 lbs. The valve was then opened, cover removed, and the cabbage drained. The contrasts in cooking conditions are summarized as follows (the $P_H$ was determined on the drained-off liquor):

COOKING CONDITIONS.

| | Pressure Cooker. | Open Kettle. |
|---|---|---|
| Time of cooking | 20 minutes in all<br>13 minutes at 15 lbs. | 45–90 minutes total |
| Temperatures maintained | 3–5 minutes at 100–121° C.<br>13 minutes at 121° C.<br>2 minutes at 121–100° C. | 45–90 min. at 100° C. |
| Reaction: Exp. II and I | $P_H$ 5.6–5.8 | $P_H$ 5.6–5.8 |
| Exp. III | $P_H$ 4.6–4.8 | |
| Contact with atmos. oxygen | In atmosphere of steam throughout cooking period. | Practically immersed in boiling water throughout cooking period. |

Guinea pigs were used as experimental animals throughout the experiments and in all cases the pigs were fed for a preliminary period of at least two weeks on the basal diet plus raw cabbage ad lib. Only those which gained consistently on this diet were selected for the experiment and those which approximated 350 gms. so far as possible. For basal diet the LaMer-Sherman combination was selected:

LAMER-SHERMAN BASAL DIET.

| | Per Cent. |
|---|---|
| Skim milk powder (Krystallak) heated two hours at 107° C. | 30 |
| Butter fat | 10 |
| Ground whole oats | 59 |
| NaCl | 1 |
| | 100 |

The cabbage was fed separately by hand and complete consumption observed of the daily allowance. The amounts used were 1 gram raw to control pigs and 5 or 10 grams cooked to experimental pigs. 5 grams cooked cabbage is equivalent to 5.4 grams raw. 10 grams pressure cooked is equivalent to 10.7 gms. raw and 10 grams open kettle cooked to 10.8 gms. raw.

Three experiments are reported herewith. Experiment I was a qualitative experiment over a twenty-day period. During this period 4 pigs were fed the basal diet plus 1 gram raw cabbage daily and two were put on basal diet without supplement. Two others were given basal diet plus 5 grams pressure cooked cabbage daily. At the end of 20 days the animals were chloroformed and autopsied. The result seemed to indicate that 5 grams cooked cabbage was very little protective as the animals showed marked scurvy symptoms. It also demonstrated the efficiency of the control diets. (See Chart, Exp. I.)

In the second experiment the control diets were repeated and four groups of experimental animals followed. These received 5 grams or ten grams daily of pressure cooked or open kettle cooked cabbage. They were kept on this diet until death ensued from scurvy and were then autopsied and the symptoms of scurvy verified. The controls on 1 gram raw cabbage per day were continued 82 days to make sure of the protection and then chloroformed and autopsied to confirm this point. (See Chart, Exp. II.)

In the third experiment three animals were placed on 10 grams pressure cooked cabbage per day plus basal diet but in this case the water in which cooking took place was acidified by diluting 40 c.c. of vinegar to 234 c.c. with water. The liquor drained from the cabbage at the end of the cooking period registered a $P_H$ of 4.6–4.8. All of these animals died of scurvy. (See Chart, Exp. III.)

A summary of the significant data connected with these three experiments is given in the following table. The growth curves are shown in the chart.

## TABLE.

*Diets (Symbols).*

A. Basal diet plus one gram raw cabbage daily.
B. Basal diet without cabbage.
C. Basal diet plus 5 grams pressure cooked cabbage daily.
D. Basal diet plus 5 grams open kettle cooked cabbage daily.
E. Basal diet plus 10 grams pressure cooked cabbage daily.
F. Basal diet plus 10 grams open kettle cooked cabbage daily.
G. Basal diet plus 10 grams pressure cooked acidified cabbage daily.

*Experiment I.*

| | Diet. | | |
|---|---|---|---|
| | *A.* | *B.* | *C.* |
| Pigs | Ave. of 4 | Ave. of 2 | Ave. of 2 |
| Days on diet | 20 | 20 | 20 |
| Wt. at beginning | 250 | 225 | 225 |
| Wt. at end | 350 | 185 | 220 |
| Autopsy findings | No scurvy | Scurvy | Scurvy |

*Experiment II.*

| | Diet. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *A.* | | *B.* | | *C.* | | *D.* | | *E.* | | *F.* | |
| Pig No. | 6 | 12 | 3 | 8 | 4 | 10 | 5 | 7 | 1 | 11 | 2 | 9 |
| Days on diet | 82 | 82 | 32 | 32 | 46 | 41 | 44 | 48 | 45 | 59 | 52 | 48 |
| Wt. at beg. | 400 | 358 | 378 | 387 | 364 | 376 | 411 | 339 | 362 | 396 | 351 | 384 |
| Wt. at end | 300 | 498 | 220 | 207 | 212 | 272 | 235 | 235 | 252 | 207 | 200 | 244 |
| Gain or loss | 100 | +140 | 158 | 180 | 152 | 104 | 176 | 104 | 110 | 189 | 151 | 140 |
| Autopsy | N[1] | N | S[1] | S | S | S | S | S | S | S | S | S |
| Adrenals | — | 640 | 410 | 453 | 439 | 302 | 509 | 481 | 350 | — | 389 | 324 mgms. |

*Experiment III.*

| | Diet *G.* | | |
|---|---|---|---|
| Pig No. | 23 | 24 | 25 |
| Days on diet | 41 | 26 | 37 |
| Wt. at beg. | 469 | 315 | 395 |
| Wt. at end | 300 | 230 | 225 |
| Loss | 169 | 85 | 170 |
| Autopsy | S | S | S |
| Adrenals | 697 | 412 | 496 mgms. |

## SUMMARY.

1. The LaMer-Sherman basal diet proved quantitatively accurate in producing death by scurvy within the periods predicted by the authors.

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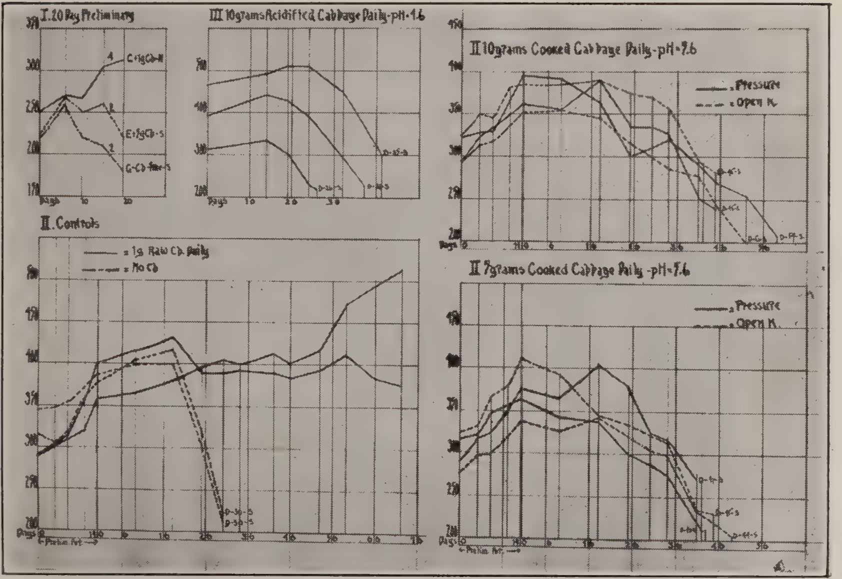
[1] "S" sign. scurvy. "N" sign. no scurvy.

2. One gram raw cabbage daily, added to the basal diet, proved completely protective against scurvy; no symptoms appearing in 82 days on the diet and the absence of symptoms in this case was confirmed by autopsy on the chloroformed survivor.

3. The methods of cookery were such as to indicate that in spite of acid reaction and exclusion of oxygen the temperatures used were sufficient to destroy enough vitamin C to make an intake of 10 grams daily inadequate for protection against the disease. In this respect the cabbage vitamin seems to be much less resistant than the orange juice or tomato juice carried form. It is of course possible that the intra-cellular oxygen of the cabbage is a factor in this difference. The results however harmonize with those of Ellis, Steenbock and Hart,[1] who showed that drying for 35 hours in an atmosphere of $CO_2$ at a temperature of 65° C. proved extremely destructive to the cabbage vitamin.



Growth curves expressed in grams. *D* indicates death by scurvy and the numeral following indicates days elapsed on diet.

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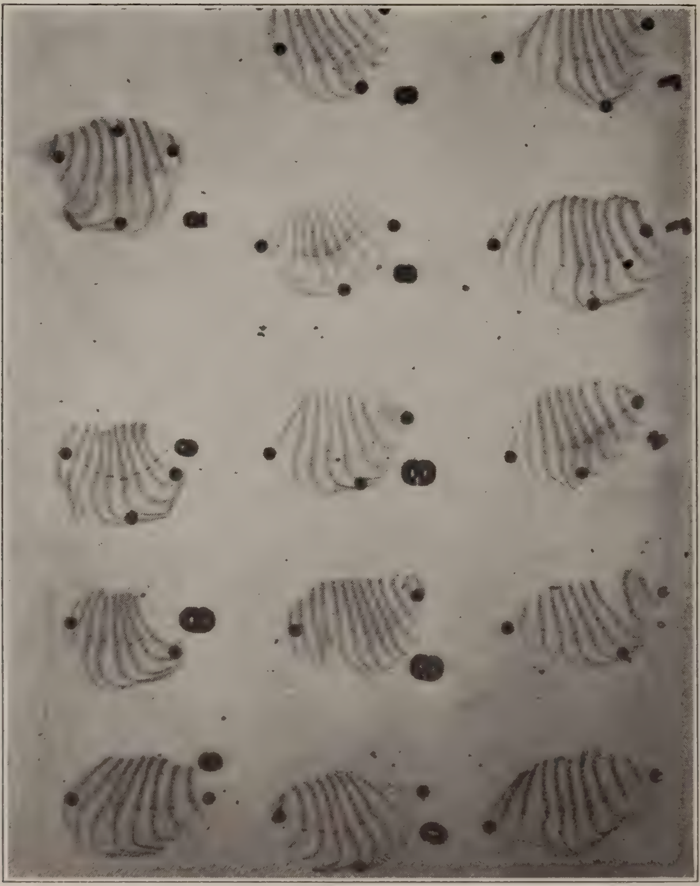
[1] *J. Biol. Chem.*, 1921, xlvi, 367.

4. At the maximum intake used, no difference was observable between the pressure cooker and the open kettle as a destructive agent.



X-rays of excised ribs of the animals of Experiment II. The numerals on the photograph have the following significance: *5* is pig 12, 82 days on basal diet plus 1 gram raw cabbage daily; *0*, pigs 3 and 8, no cabbage in diet; *4*, 5 grams pressure cooked cabbage and *3*, 5 grams open kettle cooked cabbage; *7*, 10 grams pressure cooked cabbage and *8*, 10 grams open kettle cooked cabbage daily.

### 75 (1822)

## A new sulphur-containing amino acid isolated from casein.

By **J. HOWARD MUELLER.**

[*From the Department of Bacteriology, College of Physicians and Surgeons, Columbia University, New York City.*]

In a report on a study of the cultural requirements of streptococci made last year before this society, the writer stated that a compound containing sulphur had been isolated from casein, which was apparently not related to cystine, and which seemed to be required for the growth of the test organisms. Although subsequent work has shown that this sulphur compound, when pure, is apparently not concerned in the growth of streptococci, it seemed desirable to make a study of the substance, both because of the uncertainty of the nature of non-cystine protein sulphur, and also in order to be able to effect a separation of this compound from the bacterial growth inducing factor in the amino acid fraction under investigation. While there are still many points to be cleared up in connection with the substance, perhaps enough information has been obtained to warrant a preliminary report.

There have been a number of difficulties met with in the work. The yield is very small, and probably not by any means quantitative, and further, no insoluble compounds suitable for separation have so far been found, so that purification has consisted largely in methods for the removal of impurities.

In order to obtain sufficient material for analyses, thirty pounds of commercial Argentine casein were used. Briefly, the method consists in hydrolysis with sulphuric acid, neutralization with sodium carbonate, and precipitation with mercuric sulphate solution. From the washed precipitate, freed from electrolytes, a considerable quantity of other material is removed by a second precipitation with mercuric sulphate, the sulphur compound this time remaining in the filtrate. Further purification is effected by precipitation of the filtrate, after removing electrolytes, by silver sulphate and barium hydroxide, and the compound itself is obtained from the silver filtrate, freed, of course, from Ag and Ba, by fractional crystallization, finally from dilute acetone. The yield from thirty pounds was about 10 grams.

The compound crystallizes from water, dilute alcohol and dilute acetone, in shining white microscopic plates or rosettes of rather indefinite crystal form. As a criterion of purity, amino nitrogen determinations were carried out after successive crystallizations until constant values were obtained. At this time the proportion of N to S was 2:1, and it was assumed that the preparation was pure. Analyses of this product indicated the formula $C_{11}H_{23}SN_2O_4$. Unfortunately, when most of the amino acid had been used up, it was found that the remaining material contained as an impurity a substance forming a hydrochloride relatively insoluble in concentrated HCl. Since the entire preparation had not been crystallized at the same time, and only the first portions, used for quantitative analyses, had been checked up by amino nitrogen and sulphur determinations, it is possible that the impurity was not present in the material which was analyzed. From the few tenths of a gram remaining, the insoluble hydrochloride was removed as completely as possible by dissolving in boiling HCl and allowing to crystallize on ice for several days. The filtrate, containing the sulphur compound, was freed from HCl by evaporation and by $Ag_2SO_4$, and the sparingly soluble copper salt prepared by boiling the solution with $Cu(OH)_2$ and filtering. The salt separates as microscopic, pale blue platelets. About 0.25 gram of this salt was obtained, and a single combustion and amino nitrogen determination gave the formula $C_{11}H_{21}SN_2O_4Cu$, corresponding exactly with that obtained for the amino acid in the earlier analyses. The combustion was done by the Dennstedt method, permitting the simultaneous determination of carbon, hydrogen, sulphur and copper.

The nitrogens are both in the form of amino groups, and since the solution of the compound in water is practically neutral, it is probable that two COOH groups are present. A single formol titration gave results which were slightly low, but in fair conformity with this supposition. The sulphur is not in the lead-blackening form, and is not readily split off as sulphate by boiling with acids or alkalis. The number of hydrogen atoms present suggests a straight chain compound, but no direct evidence of the structure has been obtained.

In order to rule out the possibility of the material used for analyses having been impure, it is planned to prepare another lot of about the same quantity, after which complete analytical data and a detailed account of the preparation will be published elsewhere.

When the composition and properties of the amino acid are definitely established, it will be necessary to show that it is really a primary component of protein. It is possible that the sulphur has been introduced into the molecule either from the sulphuric acid used in hydrolysis, or from the $H_2S$ used throughout the preparation. The possibility of introducing sulphur from sulphuric acid is rather remote, since the amino acid has not the properties of a sulphonic acid. However, it can be excluded only by the use of enzyme digestion or alkali hydrolysis in the primary breaking down of the protein, and this has not yet been attempted. In the preparation as outlined above, it is apparently not possible to avoid the use of $H_2S$, and this factor can be ruled out only by the elaboration of a method based on quite different principles, or by careful quantitative determinations of total sulphur throughout the various fractions, which is not likely to prove very satisfactory. It is not apparent, however, just how sulphur from $H_2S$ could be introduced into any of the known amino acids to give a compound of the above formula.

Should it be possible to exclude these sources of extraneous sulphur, this compound will probably account for a part at least of the non lead-blackening sulphur known to be present in certain proteins. The amount present may well be considerably in excess of the present yield, since the method is obviously not quantitative.

### 76 (1823)

## The inheritance of susceptibility to implants of splenic tissue in mice.

### 1. Japanese waltzing mice, albinos, and their $F_1$ generation hybrids.

By C. C. LITTLE and B. W. JOHNSON.

[*From the Carnegie Institution of Washington,
Cold Spring Harbor, N. Y.*]

The use of the terms "auto," "homio," and "hetero" transplantation has been general and of great value in the long series of experiments which have dealt with the transplantation of normal and of neoplastic tissues in vertebrates.

Following a number of experiments in this field, covering a wide range of material, it has become generally recognized by biological investigators that the closer the genetic relationship between the host and the donor of the graft tissue, the greater is the likelihood of persistent and progressive growth of an implant of tissue from one to the other.

Similarly it has been found that in the ordinary "laboratory" races of mammals, inbreeding has not been intensive enough to have produced a close degree of genetic resemblance between individuals within the race. Without this resemblance the continued growth of tissue transplants made from one animal to another is impossible. When close relatives such as parent and offspring or litter mates are picked for this interchange of implants, there is, as Loeb and others have pointed out, more chance of persistence of the implants than when unrelated animals are used. Loeb[1] has proposed the term "syngenesio-plastic transplantation" for experiments involving the close relatives referred to.

When, however, *closely inbred* races of known genetic constitution are used, results are obtained which show that the distinctions between "homio," "syngenesio," and "auto" transplantations are only relative and may be deliberately broken down by picking animals of certain definite genetic constitutions for experimentation.

Thus in animals of a closely inbred and genetically homogeneous strain of Japanese waltzing mice [already described in connection with experiments on the inheritance of susceptibility to transplanted tumors[2]], the general reactions of an individual to subcutaneous transplants of bits of its *own* spleen (autotransplants), or to bits of the spleen of *another* Japanese waltzing mouse of the same inbred race (homiotransplants) were the same. Both implants "auto" and "homio" persisted successfully, established a blood supply, and remained healthy.

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[1] Loeb, Leo, *Journ. Med. Research*, 1918, xxxix, 39–57.

[2] Little, C. C., and Tyzzer, E. E., *Journ. Med. Research*, 1916, xxxiii, 393–453.

TABLE I.

| Cross No. | Races. | Autotransplants. | | Homiotransplants. | |
|---|---|---|---|---|---|
| | | + | − | + | − |
| 1 | Japanese waltzing × Japanese waltzing | 22 | 1*a | 22 | 1*a |
| 2 | Japanese waltzing | 14 | 1*b | 0 | 15*b |
| | × albino | 16 | 1*c | 0 | 17*c |
| 3 | Japanese waltzing | 23 | 0 | 0 | 23 |
| | × $F_1$ hydrids | 33 | 0 | 33 | 0 |

+ means persistence of the implant in a healthy condition.

− means disintegration of the implant.

*a—Mouse sick and probably unable to provide adequate nourishment for either implant.

*b—One mouse negative to both auto and homio, probably due to poor operative technique or to mouse being in poor physical condition.

*c—One mouse negative to both auto and homio, probably due to poor operative technique or to mouse being in poor physical condition.

These results are tabulated in the top two lines of Table I. It should be noted that in one animal neither the autotransplant nor the homiotransplant persisted. Records show that this animal was in markedly poor physical condition and this without doubt accounts for the elimination of both implants.

When interchange of splenic implants was made between Japanese waltzing mice and unrelated albino non-waltzers, the results shown in lines 3 and 4 of Table I were obtained.

One Japanese waltzing mouse and one albino failed to support either the auto or the homio implant. This was probably due to poor technique involving infection after the operation. If these animals are subtracted from the totals we find that the *auto*-transplants in either the Japanese waltzers or the albinos are successful, while the homiotransplants from albino to waltzer or *vice versa* are uniformly unsuccessful.

This, it will be remembered, is the result ordinarily obtained in *homiotransplantation* and adds another piece of evidence to our belief that similarity in genetic constitution is essential for successful implantation of splenic tissue.

The interesting and crucial test of the correctness of the hypothesis on which the experiments were planned is found in the case of reciprocal transplants between Japanese waltzers and $F_1$ generation hybrids, formed by crossing together the waltzers and the albinos.

In this case, analogy with the tumor work and knowledge of the genetic constitution of the animals lead us to suspect that implants of waltzing mouse spleen *should* grow in the $F_1$ hybrids, while implants of splenic tissue from the hybrids *should not* persist in the waltzing mice. This was actually found to be the case, as can be seen from the two bottom lines of Table I.

The gametes of the Japanese mice were, by hypothesis, essentially equal in respect to their genetic factors. Each $F_1$ hybrid had therefore received from its waltzing mouse parent approximately the same genetic contribution. This was of such a nature as to make possible the persistence of implants coming from the Japanese mouse. (The same was found to hold true in the case of *tumors* of the Japanese mouse.)

The $F_1$ tissue which was implanted in the Japanese waltzers comes from animals approximately half of whose genetic constitution is determined by their *albino* parent. Since the Japanese mice had *not* the genetic factors which made up the albino complex, we should not expect that they could support the hybrid implants.

The results, therefore, in all three series are in complete harmony with the hypothesis advanced by Little and Tyzzer (1916), namely: that the susceptibility of any mouse to implants of foreign tumor tissue depends upon the genetic constitution of the host in its relation to the genetic constitution of the animal from which the implant was taken.

*The value of the term "syngenesiotransplantation" seems to be greatly impaired for:*

*(a) Parents (waltzers) failed uniformly to support implants of the splenic tissue of their progeny.*

*(b) Progeny ($F_1$ hybrids) grew regularly the splenic tissue of their parents.*

*There is in this case an absolute difference in the results obtained, depending upon which race is used as the host. The fact that the "genetic" relationship changes while the "pedigree" relationship remains the same, shows that the former is the important factor in determining the nature of the result.*

*The evidence obtained from the Japanese waltzing mice shows that within a closely inbred race, homiotransplants of splenic tissue may be quite as successful as autotransplants.*

The experiments further show that *in all probability susceptibility to transplants of splenic tissue depends upon the same general principles of heredity found to apply in the case of tumor tissue—namely, multiple mendelizing factors.*

### 77 (1824)

## Observations on cod-liver oil and rickets.

By **T. F. ZUCKER, A. M. PAPPENHEIMER,** and **MARION BARNETT.**

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

In view of the nearly specific action that cod-liver oil has on rickets, it is of interest to inquire into the nature of the substance conferring on it the therapeutic properties. Several attempts have been made to isolate from it materials that could be made responsible for its action. Gautier and Morgues[1] isolated the organic bases contained in the oil and separated from them two alkaloid-like substances besides the simpler aliphatic amines. Funk[2] also worked with this mixture of bases which he fractionated in various ways. None of these observers, however, have published any data on the action of the isolated material. Stöltzner[3] claims, without giving any details of his evidence, except the statement that he cured even the worst cases of rickets, that hydroxy acids confer upon cod-liver oil its pharmacological properties. Freudenberg and Klocmann[4] had expressed similar ideas and prepared calcium salts of the unsaturated acids of cod-liver oil which they used in the treatment of spasmophilia.

Wacker and Beck[5] believe that "besides other chemically not yet well characterized substances, cholesterol plays a significant rôle in the antirachitic fat soluble factor A."

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[1] Gautier and Morgues, *C. R. Acad. Sci.*, 1888, cvii, 110 and 626.

[2] Funk, *Biochem. Bull.*, 1915, iv, 365.

[3] Stöltzner, *Münch. med. Wochenschr.*, 1921, lxviii, 272.

[4] Freudenberg and Klocmann, Jahresb. f. Kinderh., 1913, lxxviii, 47; 1914, xxix, 700.

[5] Wacker and Beck, *Berl. klin. Wochenschr.*, 1921, lxxxv, 453.

With a good test object now available in the rat made rachitic on the phosphorus low diet described by Sherman and Pappenheimer, the problem of determining the point in question is much easier. The rickets of children and the experimental rickets in rats both respond in the same manner to treatment with cod-liver oil[1] and there is no reason to believe that the substance active in the two cases should not be the same.

We first isolated the crude bases according to Gautier and Morgues and found them inactive. Next the oil was hydrolyzed with sodium hydroxide and the fatty acids separated. The fatty acids, when reasonably purified were entirely inactive, although in one of the first experiments a rather impure fatty acid fraction did slightly promote calcification. The residue of unsaponifiable matter gave a marked curative action. From this the bases were again isolated and these bases obtained after hydrolysis were also inactive. From the unsaponifiable matter in solution in alcohol a goodly portion of the cholesterol was crystallized out. This cholesterol fraction also was inactive. The material freed from most of the cholesterol was now more active than before. In this manner, we obtained fractions which on being diluted with ninety parts of cotton-seed oil, which had been found to be inactive, gave a curative effect a little stronger than the original cod-liver oil. The results were controlled by both X-ray of tibia and histological examination of rib sections.

This material also contains the fat soluble factor A, as we have been able to cure with it ophthalmia produced by deficiency of fat soluble. Very recently, Steenbock, Nelson and Hart[2] have made similar other extracts of saponified cod-liver oil and reported curative effects on ophthalmia in dogs. Although there is no longer any good reason to believe that rickets is a simple fat soluble deficiency,[3] the relation of the fat soluble A factor to the curative property of cod-liver oil remains to be worked out.

To sum up, we may say that the antirachitic substance of cod-liver oil can be demonstrated in the ether soluble "unsaponifiable" fraction after alkaline hydrolysis. It is not an organic base of the type described as occurring in cod-liver oil. It is not cholesterol, but similar to cholesterol in its solubilities. The suggestion is made that it may be a sterol related to cholesterol or a cholesterol derivative. The fatty acids of cod-liver oil are entirely inactive in curing rickets.

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[1] Shipley, Park, McCollum, Simmonds and Parsons, *Jour. Biol. Chem.*, 1920–21, xlv, 343.

[2] Steenbock, Nelson and Hart, *Am. Jour. Physiol.*, 1921, lvii, 14.

[3] Pappenheimer, McCann and Hess, *Jour. Biol. Chem.*, 1921, xlvii, 395.

[4] Shipley, McCollum and Simmonds, *Jour. Biol. Chem.*, 1921, xlix, 399.

### 78 (1825)

## The distribution of inorganic phosphate of the blood between plasma and cells.

By **T. F. ZUCKER** and **MARGARET B. GUTMAN.**

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

Since Greenwald's work on the organic acid soluble phosphorus our knowledge of the various phosphorus compounds in the blood is steadily increasing and acquiring significance. The determination of the inorganic blood phosphate, however, has been very questionable, particularly in corpuscles, due to the ease with which the organic acid soluble phosphate is hydrolyzed. Any method which requires considerable time or in which the phosphate has to be precipitated, or in which the red cells are washed or otherwise manipulated, comes very gravely under suspicion of having allowed a significant amount of hydrolysis to take place.

When working only with plasma these precautions are not so necessary. Bloor's[1] figures for inorganic phosphate in the corpuscles are admittedly high. A method very well suited to estimation of inorganic phosphate is that of Bell and Doisy[2] in which the color of the blue reduction product of phosphomolybdic acid is measured as in Folin's uric acid and phenol determintion, the limiting factor, however, being the phosphate.

Our results have been briefly as follows:

When working rapidly with the Bell and Doisy method, the inorganic phosphate in the plasma and the whole blood is the same within the limit of error of the method. The few exceptions to this were traced to improper handling of the blood or too long a time elapsing before the determination. Even here the whole blood gave figures much lower than Bloor's. This shows that the phosphate ion in its relation to cells and plasma behaves differently from all the other ions studied in this respect. The chloride, for instance, is never present in cells and plasma in the same concentration. This exceptional role of the phosphate ion is, however, not so surprising when we consider the organic acid soluble phosphate, in a sense the counterpart of the chloride combined with protein.

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[1] Bloor, *Jour. Biol. Chem.*, 1918, xxxvi, 49.

[2] Bell and Doisy, *Jour. Biol. Chem.*, 1920, xliv, 55.

TABLE I.

DISTRIBUTION OF INORGANIC PHOSPHATE BETWEEN WHOLE BLOOD AND PLASMA.

| Adults. | | | Children. | | |
|---|---|---|---|---|---|
| Subject. | Whole Blood. | Plasma. | Subject. | Whole Blood. | Plasma. |
| P.G. 1 | 3.5 | 3.4 | R.M. 1 | 3.0 | 3.01 |
| P.G. 2 | 3.52 | 3.52 | R.M. 2 | 3.02 | 2.78 |
| G.M. 1 | 4.0 | 3.78 | M.E. | 4.0 | 4.01 |
| G.M. 2 | 3.98 | 3.94 | H.R. | 4.61 | 4.63 |
| J.G. | 3.1 | 2.8 | D.B. | 4.17 | 4.26 |
| E.G. | 3.38 | 3.36 | R.M. | 4.10 | 4.08 |
| T.Z. | 3.51 | 3.50 | F.B. | 3.75 | 3.49 |
| | | | M.L. | 3.16 | 3.18 |
| | | | A.A. | 4.17 | 4.12 |
| | | | A.S. | 4.25 | 4.35 |

A conclusion of practical import is that when inorganic phosphate is to be determined in blood, it is immaterial whether it is done on whole blood or plasma, providing it is done immediately by the method of Bell and Doisy.

If the colorimetric reading is made within half an hour after the blood is drawn, whole blood is no higher than plasma, but if an hour elapses the whole blood is higher by about 0.5 mg.; after several hours the difference is a milligram or more.

It seems reasonable enough to assume that if at the time the blood is drawn the phosphate ion is always present in equal concentration inside and outside of the cell, it is freely diffusible into and out of the cell when the blood circulates. Iverson[1] has shown that phosphate in organic acid soluble form can accumulate in the corpuscle at a good rate if inorganic phosphate is injected or added to blood. We would, therefore, expect that the phosphate formed by hydrolysis would diffuse out of the cells into the plasma. This seems not to be the case in the blood after it is taken from the body.

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[1] Iverson, *Biochem. Zeit.*, 1921, cxiv, 297.

TABLE II.
TO ILLUSTRATE EFFECT OF TIME AFTER DRAWING BLOOD ON THE INORGANIC PHOSPHATE.

| | | Within ½ Hr. | Within 1 Hr. | After 5 Hrs. | After 20 Hrs. |
|---|---|---|---|---|---|
| I. | Plasma | | 2.4 | 2.5 | |
| | Blood | 2.5 | 2.8 | 3.3 | |
| II. | Plasma | | 2.55 | | 2.53 |
| | Blood | 2.6 | 3.08 | | 4.02 |

These results lead to the following conception of the rôle of inorganic and organic acid soluble phosphorus in the blood: The red cell is totally permeable to the phosphate ion, *i.e.*, no "osmotic influences" control the distribution of the phosphate ion inside and outside of the cell. Phosphate ions can be taken up by the cell and stored as organic acid soluble phosphate (Iverson). This organic phosphate is hydrolyzed very easily when there is need for phosphate ion in the plasma, similar to the liver glycogen yielding blood sugar. The diffusion of the phosphate out of the cell, however (at least in vitro), is slower than its rate of formation by hydrolysis. To substantiate this view we will still have to show under what conditions inorganic phosphate can diffuse out of the cell. We have no indication so far that phosphate distribution is influenced by the $CO_2$ tension. We are now collecting data on this point, as well as on the whole subject from the point of view of the acid soluble phosphate.

### 79 (1826)

## Observations on the inorganic phosphate of blood in experimental rickets of rats.

By **M. B. GUTMAN** and **V. KNEELAND FRANZ** (by invitation).

[*From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

The work of Howland and Kramer[1] on the level of the inorganic phosphate in the blood in human rickets, and some confirmatory experiments undertaken by Hess[1] led to the conclusion that during the period of active rickets in children, the inorganic phosphate of the blood is reduced, and that during the process of cure by either sunlight or cod-liver oil, the phosphate rises again to its normal level.

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[1] Howland, J., and Kramer, B., *Amer. Jour. Dis. of Child.*, 1921, xxii, 105.

Since the experimental rickets produced in rats is comparable in most important respects to human rickets, it was thought of interest to determine whether the same changes in blood phosphate could be demonstrated in rats. In applying the work of Howland and Kramer to experimental rat rickets, we have obtained results which on the whole agree very well with those of theirs reported at a recent meeting of the Society of Biological Chemists.

Because of the small quantities of blood which can be obtained from the animals, it seemed advisable to make the determinations on whole blood rather than plasma if possible. Experiments undertaken to show the relative distribution of the inorganic phosphate in plasma and whole blood indicate that the level of the phosphate is practically the same inside and outside the cells and is maintained at a constant level from day to day. Therefore, as far as the inorganic phosphate is concerned, it is immaterial whether the determinations are made on whole blood or plasma. (By the colorimetric method of Bell and Doisy.[2])

Table I shows average figures for the inorganic phosphate in theb lood of rats on rickets-producing, normal and high phosphorus diets.

TABLE I.

INORGANIC PHOSPHATE OF RAT'S BLOOD.

*Inorganic Phosphate as Mgs. P per 100 c.c. Whole Blood.*

| Diet. | | | No. of Rats. | Determinations. | Rickets. | Blood Phosphate. | | |
|---|---|---|---|---|---|---|---|---|
| No. | % P. | % Ca. | | | | Max. | Min. | Av. |
| Norm.. | ? | ? | 55 | 43 | None in 95% | 8.2 | 5.1 | 6.2 |
| 84..... | 86 | 550 | 40 | 18 | Marked in 100% | 4.9 | 2.0 | 3.2 |
| D..... | 72 | 380 | 10 | 7 | Marked in 100% | 5.0 | 2.3 | 3.5 |
| 85..... | 160 | 550 | 7 | 2 | Sl. osteoporosis | 5.5 | 5.3 | 5.4 |
| E..... | 120 | 380 | 16 | 14 | Sl. rickets in 25% | 7.4 | 3.1 | 6.1 |
| 85 c... | 596 | 020 | 16 | 6 | Mod. atypical ric. | 7.6 | 6.0 | 6.6 |
| F..... | 596 | 015 | 12 | 9 | Mod. atypical ric. | 9.8 | 6.5 | 8.4 |
| G..... | 310 | 550 | 3 | 3 | None | 9.8 | 9.4 | 9.6 |

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[1] Hess, A. F., and Gutman, P., PROC. SOC. EXPER. BIOL. AND MED., 1921, xix, 31.

[2] Bell and Doisy, *J. Biol. Chem.* 1920, xliv, 55.

We find that in general a reduction in the inorganic phosphate of the blood runs parallel to the degree of severity of the rachitic lesions. It will also be seen that the blood phosphate of rats (on these rather specialized diets) may be greatly influenced by the level of phosphate intake. On normal diets the range is from 5.0 to 8.2, averaging 6.2, and on high phosphorus intake a very wide range from 6.0 to 9.8, averaging about 8.5 (mg. P per 100 c.c.).

The dividing line between rickets-producing diets is however sharp. Rats on diets containing 86 mg. per cent. phosphorus all develop rickets and the range of blood phosphate from 2.0 to 5.0 averages usually around 3.2. When as little as 75 mg. per cent. phosphorus is added to the diet, the rachitic lesions fail to appear and the blood phosphorus averages between 5.5 and 6.0.

The study of the blood phosphate on rats under light or cod-liver oil therapy brings up several interesting points.

TABLE II.

PREVENTION AND CURE OF RICKETS.

*Inorganic Phosphate as Mgs. P per 100 c.c. Whole Blood.*

| Diet. | No. of Determinations. | Treatment. | Rickets. | Blood Phosphate. | | |
|---|---|---|---|---|---|---|
| | | | | Max. | Min. | Av. |
| 84 | 18 | Untreated | Beading + to +++ | 4.9 | 2.0 | 3.2 |
| 84 | 10 | Mercury vapor lamp | Beading + to + | 5.4 | 2.9 | 4.1 |
| 84 | 8 | Cod-liver oil preparations | Calcification + to +++ | 5.9 | 2.4 | 3.95 |

As shown in Table II one group of rats on diet 84 (containing 86 mg. per cent. P) were treated with the light from the mercury vapor lamp as a preventive measure. In almost every case complete prevention was secured but the blood phosphorus, while distinctly above that of the controls, was nevertheless in the upper range of rachitic blood. This would seem to indicate that the rats can produce non-rachitic bone at a lower level of phosphorus intake, under the influence of light, than is possible without its presence.

Curative experiments with cod-liver oil preparations on another group of rats on the same diet show active calcification of cartilage going on while the blood phosphate is still in the rachitic range. We therefore conclude that a definite deposition of calcium salts may occur before the blood phosphorus regains its normal level. We have as yet no experiments in which the rats were carried through till the healing process was complete, but if we may draw analogies from the work of Howland and Kramer on human rickets, we ought to find that when healing is complete the blood phosphate will regain and maintain its normal level. It seems therefore that calcification is not directly controlled by the level of the blood phosphate. Experiments are, however, in progress to determine the nature of the relation between these two factors.

### 80 (1827)

## Experiments with quinidine on conduction and on the refractory period in the dog's heart.

By **ALFRED E. COHN** and **ROBERT L. LEVY.**

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

These experiments are based on the theory of fibrillation developed by Garrey and Mines on the suggestion of A. G. Mayer and recently elaborated by T. Lewis. The theory, although it illustrates best the condition known as flutter, is directly applicable also to the state of fibrillation. In the normal heart the stimulus for contraction arises at the sinus node and passes in a radial fashion to the rest of the muscle of the auricle. In flutter, and in fibrillation, there is apparently no fixed point at which the stimulus originates. It appears to be dislocated from the node but it is not yet known what activity goes on in this structure when the abnormal rhythm prevails. Instead of the usual arrangement, an excitation wave courses continuously through the muscle of the auricle, usually over a circular path about the openings of the great veins, as Lewis's experiments show. In order that a continuous circuit may be maintained it appears to be necessary (1) that the path shall have a sufficient length; (2) that the rate of passage shall be sufficiently slow, and (3) that when the stimulus returns to its starting point, the muscle is ready to receive it. It is clear, on this plan, that: (1) if the muscle mass involved is too small, a path long enough to permit the development of a circus becomes impossible; (2) if the rate of conduction is too fast, the stimulus returns to its starting point before the muscle at that point is ready for its reception; (3) if the refractory period of the muscle at the starting point is prolonged beyond the time consumed by the stimulus to make its circuit, the muscle cannot be reëxcited. The establishment and maintenance of circus movement depends then on (1) a large mass of muscle; (2) a slow rate of conduction, and (3) a brief duration of the refractory period.

When it became clear to us after trial in patients that with quinidine one could terminate the state of fibrillation in about half the individuals afflicted, we began early in 1921 to examine the nature of the activity of this drug. Certain effects which we found the drug to possess on the heart and circulation we reported to this Society at its meeting on May 18, 1921. At that time we reported that the study of its effect on the rate of conduction of impulses through the muscle was under way. We have since added to these studies an investigation of its effect on the duration of the refractory period. On these two phases of the subject we report now.

During the course of our work, Lewis's report dealing with the same phases of the subject has been published. Although the general viewpoints of the two researches are the same, they show a certain difference in that we have attempted to maintain the conditions of the experiments as nearly natural as possible and have, perhaps, in consequence of this difference in plan, arrived at somewhat different results.

These experiments were carried out on dogs, anesthetized with ether only. Artificial respiration, at constant pressure and volume, was maintained by the method of Meltzer and Auer. The chest was split and the right auricle exposed by an incision of the pericardium. A wick was sewed to the heart near the base and another near the apex of the auricular appendix. The two wicks led to non-polarizable electrodes. These electrodes formed parts of two separate galvanometer circuits which were completed by indifferent electrodes inserted in the muscle of the chest wall. Electrodes were also placed on the right fore and left hind limbs.

Records at suitable times and for different purposes were then obtainable of the usual lead 2, and of excitation at the base and at the apex of the auricle. A combination of any two records could be taken. Electrodes for sending break shocks into the auricles were permanently fixed in the auricle near the sulcus terminalis. The vagi remained undisturbed. The rate of the heart was natural. By taking simultaneous records of the excitation at the base and apex of the auricle, the distance between the electrodes being known, the rate of conduction could be calculated. The refractory period could be known exactly by obtaining and arranging in series the duration of the periods after the waves of auricular activity which the break shock terminated (Table I). These periods fall into two groups: those in which the break shocks fail to elicit a response, and those which bring about a response. The longest of the periods after which a shock fails to elicit a response is the refractory period. The break shocks were signalled by a special device to the camera and were photographed. They were likewise signalled and inscribed on the smoked record of the blood pressure. Records were made before and after injecting quinidine.

We report now on six experiments (Table II). The refractory period rose in four: 0.0042 sec. (Exp. 39), .0502 sec. (Exp. 47), 0.0336 sec. (Exp. 48), 0.0120 sec. (Exp. 46), and fell in two: 0.0101 sec. (Exp. 41) and 0.0084 sec. (Exp. 42). In two the change, one upward and one downward, was insignificant (Exps. 39 and 42). The rate of conduction fell in four (Exps. 39, 42, 46 and 47), and remained practically unchanged in one (Exp. 48). In experiment 41, the change in this rate is unknown. It is important to point out that after injecting quinidine the heart rate rose 4 times (Exps. 39, 46, 47 and 48). In experiment 46, the rate fell after subsequent injections. In two experiments (Exps. 41 and 42) the rate fell though the change was slight. In the cases in which the rate rose there was an increase in the duration of the refractory period. This result is contrary to what is anticipated, for with a rise in rate, Lewis's experiments lead one to expect a fall in this measurement. In cases in which the rate fell, there was a fall also in the length of the refractory period.

If the circus movement, as it is now believed, underlies the state of fibrillation, and if it depends on the factors which have

### TABLE I.

### Experiment 48.

Male Dog—8.0 kgm.

*Refractory Periods.*

| | Before Quinidine. | After Quinidine. 0.02 gm. (Intravenous). |
|---|---|---|
| No responses | | .0825 |
| | | .0844 |
| | .0560 | .0876 |
| | .0601 | .0904 |
| | .0605 | .0915 |
| | .0607 | .0917 |
| | .0653 | .0917 |
| | .0655 | .0941 |
| | .0663 | .0950 |
| | .0666 | .0985 |
| | .0678 | .0989 |
| | .0695 | .1000 |
| | .0699 | .1061 |
| | .0700 | .1066 |
| | .0708 | .1078 |
| | .0754 | .1079 |
| Ref. period | .0763 | .1099 |
| Responses | .0766 | .1106 |
| | .0770 | .1114[1] |
| | .0792[1] | .1123[1] |
| | .0812 | .1129 |
| | .0827 | .1135[1] |
| | .0844 | .1138 |
| | .0855 | .1145 |
| | .0859 | .1161[1] |
| | .0861 | .1194 |
| | .0868 | .1200 |
| | .0892[1] | .1207 |
| | .0960 | .1211 |
| | .0970 | .1219 |
| | | .1222 |
| | | .1228 |
| | | .1232 |
| | | .1234 |

been discussed, an action of quinidine calculated to bring this activity to an end should consist in increasing the rate of conduction and in lengthening the refractory period. In point of fact our experiments show that quinidine affects one of these factors (the refractory period) favorably, but not the other (the rate of conduction). And it affects the refractory period favorably in the doses we have used, in spite of the rise in rate which the drug

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[1] No responses.

TABLE II.
EXPERIMENTS WITH QUINIDINE.

| | Exper. 39. Male 19.9 kgm. | | Exper. 41. Male 11.6 kgm. | | Exper. 42. Male 15.5 kgm. | | Exper. 46. | | | | Exper. 47. Male 13.0 kgm. | | | Exper. 48. Male 8.0 kgm. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Before Quinidine. | After Quinidine 5 × 0.05 gm.[1] | Before Quinidine. | After Quinidine 0.05 gm.[1] | Before Quinidine. | After Quinidine 0.06 gm.[1] | Before Quinidine. | After Quinidine 0.05 gm.[1] | After Quinidine 0.07 gm.[1] Total 0.12 gm. | After Quinidine. | Before Quinidine. | After Quinidine 0.01 gm.[1] | After Quinidine 0.03 gm.[1] Total 0.04 gm. | Before Quinidine. | After Quinidine 0.02 gm.[1] |
| Heart rate | 150 | 180 | 220 | 210 | 185 | 165 | 154 | 178 | 128 | 110 | 140 | 215 | 220 | 165 | 205 |
| Refractory period | 0.0982 | 0.1024 | 0.0792 | 0.0691 | 0.1066 | 0.0982 | 0.0572 | — | 0.0692 | — | 0.0434 | 0.0812 | 0.0936 | 0.0763 | 0.1099 |
| Rate of conduction (mm. per sec.) | 1,728 | 1,351 | — | — | 1,033 | 957 | 1,121 | 1,082 | 744 | 824[2] | 2,637 | — | 2,474 | 769 | 762 |

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[1] Quinidine was injected intravenously.

[2] 12 minutes after the preceding conduction rate.

brings about. Under the circumstances, it is necessary to conclude that the effect on the refractory period is the more important. It is also a matter of interest that the dogs are not affected uniformly by the drug. It is to be recalled that a similar lack of uniformity in action exists in patients. We have, as yet, no explanation of this phenomenon. Muscular irritability is a third factor which must be considered in this connection, but a discussion of this, as well as of the details of the experiments now described and of those formerly reported, we reserve for the full account of our studies.

### 81 (1828)

## The acid base equilibrium of the blood following vigorous muscular exercise.

By DAVID P. BARR.

[*From the Russell Sage Institute of Pathology and the Second Medical Division of Bellevue Hospital, New York City.*]

Immediately following short periods of vigorous muscular work, there is a marked reduction in the bicarbonate content of the blood, a phenomenon observed by Christianson, Douglass and Haldane in 1914.[1] The initial purpose of the present investigation has been to discover what changes in the reaction and the $CO_2$ tension of arterial blood accompany the diminution in bicarbonate. For this purpose, four individuals without demonstrable organic defects but of varying grades of apparent fitness were selected for experiment. Each did on a Krogh bicycle ergometer a standard amount of exercise which consisted of the performance of approximately 3,500 kilogrammeters of work in three and a half minutes. The method employed upon the blood is that introduced by Henderson and Haggard[2] and consists in the simultaneous determination of the carbon dioxide absorption curve of blood at body temperature and the carbon dioxide content of the arterial blood as it occurs in the body. The reaction of the arterial blood is calculated from the $H_2CO_3/BHCO_3$ ratio after the formula of Hasselbalch. Arterial blood was drawn from the brachial or radial artery immediately before the exercise while the patient was resting and three minutes after the exercise had ceased. In one individual a third sample was taken eight minutes after the work was stopped.

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[1] Christianson, J., Douglass, C. G., and Haldane, J. S., *Jour. Physiol.*, 1914, xlviii, 244.

[2] Henderson, Y., and Haggard, H. W., *J. Biol. Chem.*, 1919, xxxix, 163.

In all cases there was a striking reduction in the bicarbonate content of the blood, varying in the different individuals from 10.5 to 18.8 volumes per cent. of $CO_2$. The $CO_2$ tension of the arterial blood was always lower after exercise. The diminution varied from 1.5 to 12.0 mm. The reaction of the blood was always less alkaline, in one instance assuming the very low figure of $\Gamma_H$ 7.02, practical neutrality. The reduction of $P_H$ varied from 0.09 to 0.27 in the different subjects. In the one observation eight minutes after cessation of work, the reaction of the blood, which at the end of three minutes had been $P_H$ 7.15, had resumed its original alkalinity of $P_H$ 7.30. The bicarbonate content of the blood, however, had not regained its original level and the return to normal alkalinity had been accomplished by a further reduction in $CO_2$ tension.

The effects of exercise are summarized in the table.

SUMMARY OF EFFECTS OF EXERCISE.

| Subject. | Time. | $CO_2$, Capacity of Blood at 40 mm. $CO_2$ Tension Vol. %. | $CO_2$, Content Arterial Blood Vol. %. | $CO_2$, Tension Arterial Blood mm. Hg. | $P_H$, Arterial Blood. | Remarks. |
|---|---|---|---|---|---|---|
| N. P. L. . | Before exercise...... | 45.5 | 48.10 | 45.5 | 7.27 | Athletic subject in excellent physical condition |
| | 3 min. after exercise | 34.9 | 34.66 | 39.5 | 7.18 | |
| J. McL... | Before exercise...... | 48.0 | 50.48 | 45.0 | 7.30 | Athletic subject in excellent physical condition |
| | 3 min. after exercise | 32.3 | 32.23 | 39.5 | 7.15 | |
| | 8 min. after exercise | 42.5 | 41.02 | 36.6 | 7.30 | |
| J. E...... | Before exercise...... | 41.1 | 43.84 | 43.5 | 7.25 | Normal subject with alcoholic habits |
| | 3 min. after exercise | 26.8 | 23.01 | 31.5 | 7.10 | |
| P. R..... | Before exercise...... | 44.4 | 45.75 | 41.5 | 7.29 | Acute tonsillitis, 3 days afebrile |
| | 3 min. after exercise | 25.6 | 25.84 | 40.0 | 7.02 | |

### 82 (1829)

## The effect on blood pressure of removal of portions of the spinal cord in the thoracic region.

By **R. J. BOWEN, HELEN C. COOMBS** and **F. H. PIKE.**

[*From the Department of Physiology, Columbia University, New York City.*]

The argument for the functional independence of the peripheral ganglia of the sympathetic nervous system has rested largely on the experiments of Goltz, who removed portions of the thoracic region of the spinal cord after previous transection. Two conditions should be sharply distinguished here: (1) when the transection of the spinal cord is in the lower cervical region above the level of outflow of the sympathetic fibers from the thoracic roots. This condition has been considered by Sherrington,[1] who showed that the blood pressure fell markedly on actual destruction of the spinal cord 300 days after the first transection. (2) When the spinal cord is transected in the upper thoracic region, leaving a functional connection of the medulla oblongata with the periphery through a few rami communicantes of the sympathetic system emerging with the roots of the upper thoracic nerves. Miss Yates[2] showed that systemic blood pressure fell on paralysis of the medulla oblongata some days after the transection of the spinal cord. It remains to determine the actual effect upon systemic blood pressure of removal of portions of the spinal cord below the level of transection after an interval of recovery.

Cats were used in our experiments. The spinal cord was transected under aseptic conditions at varying levels from the second to the ninth thoracic, and the animal allowed to recover. Some days afterward, the animal was again anesthetized and the systemic blood pressure recorded from a cannula in one carotid artery. A mercury manometer was used. In one cat, in which the level of transection was just below the fifth thoracic root, the mean level of blood pressure was 114 millimeters of mercury six days after the transection. The blood pressure remained at 78 millimeters after removal of a section of the spinal cord eight centimeters long below the level of the first transection. Similar results were obtained in other animals. A greater fall of blood pressure followed removal of the lumbar and sacral portions of the spinal cord, below the level of outflow of thoracic sympathetic fibers. Any interference with the function of the upper portion of the thoracic cord or the medulla oblongata was always followed by an extreme depression of blood pressure, whatever the condition of the spinal cord. The results indicate the functional dependence of the peripheral ganglia of the sympathetic system upon the central system.

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[1] "The Integrative Action of the Nervous System," 1906, pp. 241–243.

[2] *American Journal of Physiology*, 1921, lvii, 68.

### 83 (1830)

## A simple quantitative precipitation reaction for syphilis.

By **R. L. KAHN.**

[*From Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.*]

The proposed method possesses the following three advantages over the Meinicke,[1] Sachs and Georgi,[2] and Dreyer and Ward[3] (Sigma) precipitation reactions.

1. The diluted antigen possesses considerable stability, rendering it unnecessary to dilute fresh antigen before using in the tests.
2. The strongly positive serums show, in most cases, spontaneous precipitation and the test as a whole is completed after 3 hours incubation in the water bath.
3. The precipitates can be easily distinguished with the naked eye.

*The Method.*—The antigen is prepared according to Neumann and Gager.[4] After extracting the dried heart muscle with ether and drying as indicated by these authors, 5 c.c. of absolute alcohol is added to each gram of material.

The alcoholic extraction is carried on for about 10 days in the ice box. It is then filtered and fresh alcohol added to the extent of the amount filtered off. The second extraction is carried out for about a month in the ice box. Both filtrates may be used as antigens.

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[1] *Berl. klin. Wchnschr.*, 1918, iv, 83; *Muench. med. Wchnschr.*, 1918, lxv, 1279, and ibid., 1919, xxxiii, 932.

[2] *Med. Klinik*, 1918, xxxiii, 805.

[3] *Lancet*, 1921, xix, 956, Old Series C.C.

[4] *J. Immun.*, 1917, ii, 573.

Add rapidly three parts of salt solution to 1 part of antigen. The mixture should be opalescent and but slightly milky. No precipitate should form after standing over night at room temperature. Antigens conforming with these requirements have been kept for 2 weeks without any apparent change in antigenic properties.

Add 0.1 c.c. of antigen to 0.3 c.c. of clear inactivated serum and incubate in the water bath. The strongly positive sera show immediate clouding and, within 5 min., visible precipitation. For uniformity, the tests are read after 1 hour and 3 hours incubation. Those showing marked precipitation at the end of 1 hour are considered strongly positive; marked precipitation at the end of 3 hours are considered positive; weak precipitation, weak positive and questionable, doubtful positive.

Of 1,119 comparative tests carried out with the precipitation and Wassermann reactions as performed in this laboratory, 98 per cent. showed agreement. It is hoped that this test will form an important supplement to the Wassermann test.

### 84 (1831)

## A simple quantitative precipitation reaction for syphilis—micro procedure.

By **R. L. KAHN.**

[*From Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.*]

The precipitation reaction presented in the preceding paper may be employed also as a microscopic procedure. Prepare hanging drop preparations in the usual manner by mixing a small drop of serum with the same amount of antigen. Read results after 1 and 3 hours incubation at 37.5° C. Those sera showing precipitation give the appearance of clumps of minute globules, while the negative sera appear homogeneous. This procedure, however, requires much experience and care in its manipulation.

The following micro-method is, in our experience, far simpler to execute and is recommended: Pipette 0.03 c.c. quantities of serum in narrow agglutination tubes and add 0.02 c.c. of antigen to each tube. Incubate in water bath and read as in the case of the regular quantities described in the preceding paper. Although the total quantity employed in this test is only about 1/10 of the original one, the precipitates can nevertheless be seen with the naked eye. We would, however, recommend this test only in cases where it is extremely difficult or impossible to obtain larger quantities of serum.

### 85 (1832)

## Contribution to the chemico-pharmacodynamic relationship of atropine and homatropine.

By DAVID I. MACHT.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

The classical researches of Ladenburg on the structure of atropine and the synthesis of various tropeins led almost immediately to a wide therapeutic application of homatropine as a mydriatic. Inasmuch as the mydriatic action of atropine is known to be through the parasympathetic nerve-mechanism of the eye, namely, the paralysis of the parasympathetic endings of the oculomotor nerve, it has been generally assumed that the mydriatic action of homatropine or tropin-mandelate was of exactly the same nature. An examination of experimental data on the subject, however, gives no proof to support this assumption. In the present investigation, the author became interested in the pharmacology of homatropine in connection with a study of mandelic acid. This acid is closely related to benzaldehyde and indeed can be readily prepared from the latter by treatment with hydrocyanic acid and water. Inasmuch as the author has already shown that benzaldehyde possesses the antispasmodic or relaxant properties on smooth muscle which are exhibited by benzyl alcohol and certain benzyl esters, it was thought possible that the action of homatropine may be exerted, at least partially, directly on smooth muscle itself. A series of experiments tended to corroborate his view. In the first place, the action of homatropine on other parasympathetic nerve endings, such as the vagus terminals in the heart, is very much weaker than that of atropine. Whereas a small dose of atropine completely paralyzes the vagus endings in the heart, so that electrical stimulation, even of great intensity, fails to inhibit the heart-beat, it takes about ten times as much homatropine to produce the same effect. In the second place, when such experiments on the vagus are performed it is interesting to note that injections of homatropine are followed by a fall in blood pressure and a vasodilatation which is obvious even to the naked eye, when the intestines are inspected. In the third place, a comparative study of atropine and homatropine on uterine, intestinal and other smooth muscle in vitro showed that the relaxant effect of homatropine was much greater than that of atropine. Furthermore, two other esters of mandelic acid which have been employed therapeutically were also found to exhibit marked antispasmodic effects on smooth muscle. These are antipyrin-mandelate, or *tussol* and eucain-mandelate, or *euphthalmin*. While antipyrin itself and eucain itself have very little effect on smooth muscle the mandelic acid esters of these substances were found to be markedly antispasmodic or depressant for that tissue. Finally, the author has prepared and studied the simple salts of tropic acid and mandelic acid themselves and found that whereas sodium tropate has little or no effect on the contractions and tonus of smooth muscle, sodium mandelate exhibits a relaxant action and when used in strong solution (5 to 10 per cent.), it was found to produce a mydriasis when instilled into a rabbit's eye. The above data indicate pretty conclusively that the mydriatic effect of homatropine is not entirely due to a paralysis of the parasympathetic innervation but is probably, at least in part, to be explained by direct action of the drug on the muscle cells themselves. Further work on the subject is in progress. The author is investigating the properties of benzyl mandelate. This investigation is supported, in part, by a fund from the Research Council of the American Pharmaceutical Society.

### 86 (1833)

## Effect of prostatectomy on integration of muscular movements of the white rat.

By **D. I. MACHT** and **J. L. ULRICH.**

*[From the Pharmacological and Physiological Laboratories, Johns Hopkins University, and the Brady Urological Institute, Baltimore Md.]*

An attempt to study the effect of prostatectomy on muscular coördination and efficiency was made by means of the so-called "rope problem." White albino rats are trained to walk across a room over a tightly stretched rope starting from a platform at one end and ending at another platform on which food is placed at the other end of the room. This problem or method of study is an excellent one for the development of muscles and training of their coördination. The animals at first cannot cross the rope at all and slip off it repeatedly, hanging by the front legs. After fifty trials, however, they learn to coördinate their movements and eventually can run over the entire length of the rope rapidly and without swaying or slipping off. After such a prolonged trial one notices a marked improvement in the tonus and strength of the entire musculature of the animals. In the present investigation the effect of prostatectomy was studied on the coördination of muscular movements. Two sets of experiments were performed. In the one group of rats, the animals were trained on the rope until they mastered the problem perfectly. They were then prostatectomized and allowed to recover. After recovery the animals were quickly retrained and it was found that there was no evident change in the integration of muscular movements produced by the extirpation of the prostate glands. In the second set of experiments, another group of rats were prostatectomized after seven trials on the rope before they had completely mastered the problem. The animals were allowed to rest and after recovery from the operation training on the rope was again begun. It was soon noticed that this group of rats learned to run across the rope very poorly and indeed after even a much longer period of training than the first group of rats (80 trials) in this second group of animals rhythmic progression was very poorly established. The progression of the animals was much slower and more difficult. The muscles showed frequent tremblings and especially the muscles of the hind legs showed marked weakness. This was not exhibited by control rats in which laparotomy was performed but in which the prostates were not excised. In this second group of animals, futhermore, a marked improvement in muscular efficiency was manifested after feeding of dried prostate and certain other glands, which will be described more fully in the complete paper to appear in the *Journal of Urology*.

### 87 (1834)

## Vitamin A in oranges.

By **THOMAS B. OSBORNE** and **LAFAYETTE B. MENDEL.**

[*From the Laboratory of the Connecticut Agricultural Experiment Station and the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

In an earlier paper dealing with citrus fruits[1] we stated that preliminary tests indicated that dried orange juice contains some vitamin A. This conclusion was based on the fact that when the equivalent of 10 c.c. juice was furnished daily to rats on a diet practically devoid of vitamin A, the symptoms which characteristically ensue upon such a dietary régime did not develop within the period of 190 days during which our observations continued. For example, the now well known ophthalmia[2] was either cured or averted.

A reinvestigation of the subject has substantiated our earlier conclusion. In a number of rats maintained on a diet consisting of casein, starch, lard and salt mixture,[3] together with 0.2 gm. of dried brewery yeast as a source of vitamin B, the characteristic ophthalmia associated with a lack of vitamin A was completely cured within a few days after the daily administration of either 10 c.c. of fresh orange juice or the same amount of juice desiccated, admixed with starch, in a current of hot air. Five c.c. of juice sufficed to cure the ophthalmia but a larger quantity appeared to be necessary to secure restoration of growth. Inasmuch as Cooper[4] has reported the presence of vitamin A in orange peel, special precaution was taken in our work to avoid contamination of the juice with the latter.

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[1] Osborne and Mendel, *J. Biol. Chem.*, 1920, xlii, 465.

[2] Osborne and Mendel, *J. Am. Med. Assn.*, 1921, lxxvi, 905.

[3] Osborne and Mendel, *J. Biol. Chem.*, 1919, xxxvii, 572.

Owing to the comparative richness of orange juice in carbohydrates, so that 10 c.c. represent a not inconsiderable intake of non-protein calories, it is important that the proportion of protein and essential salts in the rest of the ration be large enough to promote growth at the normal rate. The data now available from animal feeding experiments indicate the presence of vitamins A, B, and C in the orange and the possibility of conserving them, in part at least, undeteriorated by suitable processes of desiccation. With respect to the proportions of these different vitamins present our experiments indicate that volume for volume orange juice is as rich as is milk in vitamin B, but somewhat less rich in vitamin A. According to the data furnished by Givens and McClugage,[5] orange juice is much richer than milk in vitamin C.

### 88 (1835)

## Studies in experimental plethora in dogs and rabbits.

By **E. B. KRUMBHAAR** and **A. CHANUTIN.**

[*From the John Herr Musser Department of Research Medicine, University of Pennsylvania, and the Laboratories of the Philadelphia General Hospital, Philadelphia, Pa.*]

The object of the present communication is to present the functional changes produced by repeated transfusions in the blood-making and blood-destroying apparatus, and in metabolism, and the structural changes in the viscera of dogs and rabbits. In an attempt to throw further light on the relation of the spleen to blood formation and blood destruction, we first studied the effect of splenectomy in artificial plethora, and tried to find evidence of increased enzyme action in the spleen removed at a time when blood was being destroyed in greatly increased quantities. Not only were these efforts barren of results, but it was also found that our knowledge of the changes caused by the artificial induction of plethora was in itself meager.

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[4] Cooper, PROC. SOC. EXPER. BIOL. AND MED., 1921, xviii, 243.

[5] Givens and McClugage, *Am. J. Dis. Child.*, 1919, xviii, 30.

The effects of repeated transfusions of blood on the blood-destroying and forming apparatus of normal and splenectomized dogs and rabbits have been described. An anemia which developed in two splenectomized dogs during a plethora despite continued blood transfusions has also been studied.

The decrease or absence of reticulocytes from the blood stream during plethora and their increase during anemia is evidently due to depression and activation of bone marrow activity. The response of the bone marrow is not immediate upon the onset of anemia, but is developed after several days.

Blood volume studies have served to emphasize the constancy of plasma volume under extreme experimental conditions, and the adaptability of the circulatory system to large increases in total blood volume.

Blood destruction and elimination as measured by urobilin excretion is greatly increased during the induction of plethora, but still more so during "plethoric anemia."

Despite intravenous introductions of large quantities of nitrogen in the form of whole blood, the total nitrogen, urea and ammonia in the urine and feces is not raised appreciably for some time after the onset of plethora. The normal organism is apparently able to store large quantities of blood or its decomposition products. Upon the onset of a "plethoric anemia," there is an increase in urinary total nitrogen, urea and ammonia excretion, which is lowered during the course of the anemia. Albuminuria is also found at this time. Other nitrogenous constituents and phosphates show no striking changes.

Blood pigment, chiefly in the form of hemosiderin, is deposited in enormous quantities in the spleen, liver, lymphnodes and bone marrow. It occurs chiefly in phagocytes, though in late stages large extracellular masses are found. Increased pigment deposition can still be found several months after transfusions have been stopped.

Phagocytes containing erythrocytes are only occasionally found in the "acute" cases, but their occurrence may have been greatly masked by the coexistent congestion.

In splenectomized animals the tendency to "plethoric anemia" is much more apparent, although a direct connection between the two events has not been established.

In splenectomized animals pigment-bearing phagocytes are especially prominent in the liver, although lymphnodes and bone marrow apparently share in the extra work caused by the absence of the spleen. Lymphnodes with some of the characteristics of hemolymphnodes have been found in various localities in all animals that had been made plethoric.

In rabbits, blood pigment is deposited in the organs in large amounts, but the picture and the experiment has in our hands been constantly complicated by early fatal intravascular agglutination and thrombosis. In the rabbit, as in human hemochromatosis, the pigment is found in 2 forms: Hemosiderin granules, and smaller, dark spicules that do not react to the usual iron stains (probably hemofuscin). The latter pigment is also found seeded through the cells of the liver parenchyma.

## 89 (1836)

## Changes in total peripheral resistance during experimental shock.

By DONALD D. FORWARD and LOUIS J. PERME.

[*From the Physiological Laboratory of Western Reserve University Medical School, Cleveland, Ohio.*]

The question as to whether the peripheral resistance is increased or decreased in experimental shock has been submitted to repeated investigations, but with contradictory results.[1] On the basis of changing contours of the aortic pressure curves found during the course of experimental shock, Wiggers[1] came to the conclusion that a reduced peripheral resistance obtained early in shock. Apparently contradictory results were however soon reported by Erlanger, Gasser and Gesell [1] who employed, in modified form, the procedure described by Bartlett[2]—a method which measures essentially the rate of saline inflow into the main artery of an organ or limb temporarily isolated from the rest of the arterial circulation. In view of the investigations of Dale and Richards[3] who found that the normal reactions of capillaries are not maintained when an organ is perfused with saline solution, whereas the reactions of the arterioles are retained, it seemed not impossible that Erlanger, Gasser and Gesell by their method tested largely the reaction of the peripheral arterioles in shock, whereas the optical curves analyzed by Wiggers determined peripheral resistance changes due in part also to alterations in the caliber of the capillaries, viscosity of blood, etc. Since evidence has accumuated that the capillaries are particularly affected in shock it is quite possible that their dilation might decrease the total resistance early in shock in spite of the fact that a contraction of their supplying arterioles occurs. If this be true the discordant finds of Wiggers and Erlanger et al. would be explained.

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[1] For recent review of literature cf. Wiggers, *Amer. J. Physiol.*, 1918, xlvii, 498; Erlanger, Gesell and Gasser, *Amer. J. Physiol.*, 1919, xlix, 103.

[2] Bartlett, *Jour. Exp. Med.*, 1912, xv, 415.

At the suggestion of Doctor Wiggers, we therefore attempted to determine by direct methods how the *total peripheral resistance* behaved during the course of experimental shock—particular attention being directed to early phases. To do this we employed the method described by Cope[4] which essentially determines the rate that the animal's own blood flows through a limb temporarily isolated from the rest of the arterial circulation. The results obtained by this method at various times during the course of an experiment were related not only to changes in mean blood pressure and heart rate but to the contours of the optically recorded carotid pulse as well. After preliminary ligation of the pelvic vessels required for the procedure described by Cope, shock was induced by the "gastric massage" method described by Henderson and Haggard.[5]

*Results.*—Thirteen experiments were carried out on anesthetized dogs. Comparison of the optical curves before and after ligation of the pelvic vessels revealed no changes such as were found by Wiggers in the initial stages of shock. Arterial blood pressure sometimes declined temporarily but recovery to or above normal was usually prompt. Control tests of the total peripheral resistance indicated that it usually increased for a time.

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[3] Dale and Richards, *Jour. Physiol.*, 1918, lii, 144.

[4] Cope, *Amer. J. Physiol.*, 1911, xxix, 137.

[5] Henderson and Haggard, *J. Biol. Chem.*, 1918, xxxiii, 136.

Shortly after opening the abdomen and beginning of the gastric manipulation the total resistance showed pronounced changes. In a few cases (especially those in which there had been a previous hemorrhage), the total resistance was found to increase, for a time. The optical curves in such cases showed no essential variations, however. In the majority of cases, however, the total resistance as measured by the Cope method decreased at once and the optical curves showed typical changes interpreted as characteristic of low peripheral resistance.

During the progressive stages of shock, *i.e.*, where mean arterial pressure begins to fall, wide fluctuations in resistance were found by the Cope method, confirming observations of Erlanger, Gasser and Gesell. In those experiments, however, in which the total resistance was initially decreased, it continued below normal.

We believe, therefore, that direct proof has been supplied that the early changes in contours of the arterial pressure curves during shock are associated with a reduced peripheral resistance. Taken in conjunction with the observations of Erlanger, Gesell and Gasser that the arterioles at this time are constricted these results lend support to the idea that the point of vaso-relaxation in shock is in the capillaries rather than the arterioles.

### 90 (1837)

## Experimental plumbism: therapeutic efficiency of some agents and comparative toxicity of other metals.[1]

By **P. J. HANZLIK, MARY McINTYRE** and **ELIZABETH PRESHO.**

[*From the Departments of Pharmacology, Leland Stanford Junior University, San Francisco, Cal., and Western Reserve University, Cleveland, Ohio.*]

Experimental chronic lead poisoning was produced by feeding metallic lead in the form of bullets to pigeons. The symptoms are characterized by a prompt loss of body weight and appetite, gradual depression, loss of equilibrium, diarrhea, increased crop peristalsis with regurgitation of contents, wing drop (anatomically corresponding to drop-wrist in man), paralysis of legs, marked emaciation and death at the end of 21 days (mean). At autopsy, the principal lesions observed are marked atrophy of the skeletal musculature everywhere, and sometimes darkening of mucosa of the large intestine and cloaca; unabsorbed lead bullets, if any, being found in the gizzard.

The results on 63 animals to date may be briefly summarized as follows: The lethal dose of lead was found to be about 0.16 gm. per kilo; time of death in fatal cases was 21 days; time of recovery in survival cases was 26 days to 8 months; lead absorbed in fatal cases was 85 per cent.; concentration of lead in tissues of fatal cases was about 0.075 per cent.; and the current of lead, about 0.0103 gm. per kilo per diem for 83 days to 0.02 gm. per kilo per diem for 25 days; the maximal loss of body weight in fatal cases was 40 per cent., in surviving pigeons 8 per cent.; the first appearance of loss of body weight in all animals was demonstrable at end of 2 to 4 days after administration and the greatest loss of body weight occurred at the end of 20 days; loss of body weight proceeded or was parallel with diminished food intake apparently due to loss of appetite from sickness; the daily food intake was 3.9 gm. in fatal cases and 18 gm. in survival cases; the normal food intake being 23 gms.

All of the above factors were beneficially influenced by the administration of sodium iodide in food and water and magnesium sulphate and calcium sulphide in food, while sodium chloride administered in the same way was not beneficial.

The following lead salts administered in doses whose lead content was 2 to 13 times that of the minimal fatal dose of metallic lead were non-toxic: lead chloride, lead iodide, lead acetate and lead sulphide.

Of the following metals used in 2 1/5 times the dosage of the minimal fatal dose of lead, namely, zinc, copper, tin, bismuth, iron and cadmium, only cadmium, bismuth and zinc were toxic, but not fatal during a period of 27 days of observation. Therefore, lead is decidedly more toxic, and plumbism is more or less a specific toxicity in the sense that symptoms occur promptly and in a striking manner, the motor effects and fatalities being absent with cadmium, bismuth and zinc.

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[1] Presented at the thirty-first meeting of the Pacific Coast Branch, December 15, 1921.

# SCIENTIFIC PROCEEDINGS.

## ABSTRACTS OF COMMUNICATIONS.

## One Hundred Twenty-first Meeting

*University and Bellevue Hospital Medical College, February 15, 1922.*
*President Wallace in the chair.*

### 91 (1838)

### A method for the rapid determination of urea in minute amounts of blood.

By **ISRAEL S. KLEINER.**

[*From the Department of Physiological Chemistry of The New York Homoeopathic Medical College and Flower Hospital, New York City.*]

This method involves the digestion of urea by urease (Marshall-Van Slyke), the precipitation of the proteins (Folin-Wu), the direct Nesslerization of the filtrate (Myers) and determination of the color in the micro-colorimeter previously described.[1] For this color comparison a wedge, containing 1 per cent. potassium dichromate, mounted on a deep yellow ground-glass plate, is used.

The technique is as follows: A 0.2 c.c. pipette is rinsed with 20 per cent. potassium oxalate solution. The residual fluid is blown out well and 0.2 c.c. of blood is drawn up from the pricked finger or ear-lobe and discharged into a small test-tube. The pipette is then rinsed twice with exactly 0.2 c.c. of water and the washings are added to the blood. Three or four milligrams (knife-point) of powdered urease are now added to the blood and, after shaking, a stopper is inserted and the tube is kept at 50° for 10 minutes or at room temperature for 30 minutes or longer. Then 1.0 c.c. of water is added, followed by 0.2 c.c. of 10 per cent. sodium-tungstate solution and 0.2 c.c. of 2/3 N sulphuric acid.

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[1] Kleiner, *Journal A. M. A.*, 1921, lxxvi, 172.

The mixture is immediately shaken and, after the precipitate has darkened, it is filtered into another small test-tube, using a 2.5–3 cm. funnel and small thin filter paper. With a dry 1 c.c. pipette, graduated in 1/100ths, a definite volume of the filtrate is discharged into one of the 5 c.c. graduated test-tubes with which the micro-colorimeter is provided. It is convenient to take 0.5 c.c. but one need not wait for this amount to filter through. Two volumes of water are added and one volume of Nessler's solution (Bock-Benedict formula diluted 1 : 5). After thorough mixing the tube is placed in the micro-colorimeter, and matched to the standard "nitrogen" wedge, described above. The reading is now made on the scale and the amount of urea nitrogen per 100 c.c. blood found directly by consulting Table I. If the color is too deep,

TABLE I.

DILUTION,—1 VOL. SOLUTION : 2 VOLS. WATER : 1 VOL. NESSLER'S SOLUTION.

| Reading. | Mg. N per 100 c.c. | Reading. | Mg. N per 100 c.c. | Reading. | Mg. N per 100 c.c. | Reading. | Mg. N per 100 c.c. |
|---|---|---|---|---|---|---|---|
| 10 | 10 | | | | | | |
| 11 | 10 | 36 | 22 | 61 | 34 | 86 | 45 |
| 12 | 11 | 37 | 22 | 62 | 34 | 87 | 45 |
| 13 | 11 | 38 | 23 | 63 | 34 | 88 | 46 |
| 14 | 12 | 39 | 23 | 64 | 35 | 89 | 46 |
| 15 | 12 | 40 | 24 | 65 | 35 | 90 | 47 |
| 16 | 13 | 41 | 24 | 66 | 36 | 91 | 47 |
| 17 | 13 | 42 | 25 | 67 | 36 | 92 | 48 |
| 18 | 14 | 43 | 25 | 68 | 37 | 93 | 48 |
| 19 | 14 | 44 | 26 | 69 | 37 | 94 | 49 |
| 20 | 15 | 45 | 26 | 70 | 38 | 95 | 49 |
| 21 | 15 | 46 | 27 | 71 | 38 | | |
| 22 | 15 | 47 | 27 | 72 | 39 | | |
| 23 | 16 | 48 | 27 | 73 | 39 | | |
| 24 | 16 | 49 | 28 | 74 | 40 | | |
| 25 | 17 | 50 | 28 | 75 | 40 | | |
| 26 | 17 | 51 | 29 | 76 | 40 | | |
| 27 | 18 | 52 | 29 | 77 | 41 | | |
| 28 | 18 | 53 | 30 | 78 | 41 | | |
| 29 | 19 | 54 | 30 | 79 | 42 | | |
| 30 | 19 | 55 | 31 | 80 | 42 | | |
| 31 | 20 | 56 | 31 | 81 | 43 | | |
| 32 | 20 | 57 | 32 | 82 | 43 | | |
| 33 | 20 | 58 | 32 | 83 | 44 | | |
| 34 | 21 | 59 | 33 | 84 | 44 | | |
| 35 | 21 | 60 | 33 | 85 | 44 | | |

five volumes of water are added and, after mixing, a comparison is again made, the result this time being found by consulting Table II. These tables cover a range from 10 to 100 mg. of urea N per 100 c.c. blood.

TABLE II.

Dilution,—1 Vol. Solution : 7 Vols. Water : 1 Vol. Nessler's Solution.

| Reading. | Mg. N per 100 c.c. | Reading. | Mg. N per 100 c.c. | Reading. | Mg. N per 100 c.c. |
|---|---|---|---|---|---|
| 20 | 31 | | | | |
| 21 | 32 | 46 | 62 | 71 | 92 |
| 22 | 33 | 47 | 64 | 72 | 94 |
| 23 | 34 | 48 | 65 | 73 | 95 |
| 24 | 36 | 49 | 66 | 74 | 96 |
| 25 | 37 | 50 | 67 | 75 | 97 |
| 26 | 38 | 51 | 68 | 76 | 98 |
| 27 | 39 | 52 | 70 | 77 | 99 |
| 28 | 41 | 53 | 71 | 78 | 100 |
| 29 | 42 | 54 | 72 | | |
| 30 | 43 | 55 | 73 | | |
| 31 | 44 | 56 | 74 | | |
| 32 | 46 | 57 | 76 | | |
| 33 | 47 | 58 | 77 | | |
| 34 | 48 | 59 | 78 | | |
| 35 | 49 | 60 | 79 | | |
| 36 | 50 | 61 | 80 | | |
| 37 | 51 | 62 | 82 | | |
| 38 | 53 | 63 | 83 | | |
| 39 | 54 | 64 | 84 | | |
| 40 | 55 | 65 | 85 | | |
| 41 | 56 | 66 | 86 | | |
| 42 | 58 | 67 | 88 | | |
| 43 | 59 | 68 | 89 | | |
| 44 | 60 | 69 | 90 | | |
| 45 | 61 | 70 | 91 | | |

The tables represent the averages of many determinations made on sunny and on cloudy days. This minimizes the slight differences due to variations in intensity of light.

Results obtained with this method agree closely with analyses of the same specimens by the aeration method in which 2 c.c. of blood were used. The micro method may be performed easily in 20–30 minutes with sufficient accuracy for clinical purposes.

### 92 (1839)

## The vitamine requirements of certain yeasts and bacteria.

By **LOUIS FREEDMAN**[1] and **CASIMIR FUNK.**

[*From the Laboratory of Biochemistry, College of Physicians and Surgeons, Columbia University, and the Research Laboratory of H. A. Metz, New York City.*]

In discussing the nutritional requirements of the microörganisms, we cannot overlook the important rôle that the vitamines play. The identity of the vitamine that influences the growth of the lower organisms is still an open question. The bulk of evidence, however, points strongly to the conclusion that this substance is distinct from vitamine B, although it is closely related to it; and our results lead us to draw the same conclusion.

Preliminary experiments with beef-heart infusions, peptone, and autolyzed yeast solutions, have shown us that these media contain substances which have a comparable growth-stimulating action on hemolytic streptococci and yeast cells. Thus a beef-heart infusion gives a profuse growth when inoculated with streptococci, whereas this medium, when decolorized by boiling with 2 per cent. of its weight of norit charcoal, loses its growth-stimulating activity, even on addition of a glucose-salt solution. This confirms some of the results obtained by Mueller. When 1 per cent. peptone or autolyzed yeast solutions are added to the decolorized infusion, the medium again becomes favorable for the growth of streptococci. Analogous results were obtained with these substances on the growth of yeast cells.

As it was more desirable to separate these activating substances from the bulk of impurities with which they are associated in their natural media, we subjected beef-heart infusions and autolyzed yeast solutions to fractional adsorption by means of fuller's earth and norit according to the method of Funk and Dubin. These authors have shown that at least two different substances can be separated from autolyzed yeast by means of fractional adsorption with fuller's earth. By this method it is now possible to separate the vitamine active for yeast growth, which has been provisionally called "vitamine D," from that of the anti-beriberi or B vitamine.

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[1] The data in this paper was taken from a dissertation to be presented by Louis Freedman in partial fulfillment for the degree of Doctor of Philosophy, in the Faculty of Pure Science, Columbia University.

TABLE I.

EFFECT ON GROWTH OF YEAST CELLS AND STREPTOCOCCI OF SHAKING OF AUTOLYZED YEAST WITH FULLER'S EARTH AND NORIT.

| No. | | Yeast Growth. | Bacterial Growth. |
|---|---|---|---|
| | Fuller's Earth: | | |
| 1. | Autolyzed yeast (5% solution) | 12.5 | ++ |
| 2. | " " shaken with 50 grams per liter | 9.5 | ++ |
| 2(*a*). | Baryta extract of fuller's earth (from 2) | 5.5 | ++ |
| 3. | Autolyzed yeast (filtrate from 2) shaken with 100 grams per liter | 5.0 | − |
| 3(*a*). | Baryta extract of fuller's earth (from 3) | 4.0 | ++ |
| 4. | Autolyzed yeast (filtrate from 3) shaken with 100 grams per liter | 0.5 | − |
| 4(*a*). | Baryta extract of fuller's earth (from 4) | 0.0 | − |
| | Norit: | | |
| 5. | Autolyzed yeast shaken with 50 grams per liter | 10.5 | + |
| 5(*a*). | Acetic-acid extract of norit (from 5) | 4.0 | ++ |
| 6. | Autolyzed yeast (filtrate from 5) shaken with 100 grams per liter | 3.0 | − |
| 6(*a*). | Acetic-acid extract of norit (from 6) | 3.0 | + |
| 7. | Autolyzed yeast (filtrate from 6) shaken with 100 grams per liter | 0.0 | − |
| 7(*a*). | Acetic-acid extract of norit (from 7) | 0.0 | − |

TABLE II.

SHOWING EFFECT ON GROWTH OF YEAST CELLS AND STREPTOCOCCI OF FRACTIONAL SHAKING OF BEEF-HEART INFUSIONS WITH FULLER'S EARTH AND NORIT.

| No. | | Yeast Growth. | Bacterial Growth. |
|---|---|---|---|
| | Fuller's Earth: | | |
| 1. | Beef-heart infusion 1 c.c. [equiv. to ($\frac{2}{5}$ gm.) beef-heart] | 12.0 | ++ |
| 2. | Beef-heart infusion shaken with 50 grams per liter | 3.0[1] | ++ |
| 2(*a*). | Baryta extract of fuller's earth (from 2) | 1.0 | + |
| 3. | Beef-heart infusion (filtrate from 2) shaken with 100 grams per liter | 0.0 | − |
| 3(*a*). | Baryta extract of fuller's earth (from 3) | 1.0 | + |
| | Norit: | | |
| 4. | Beef-heart infusion shaken with 20 grams (2%) per liter | 0.0 | + |
| 4(*a*). | Acetic-acid extract of norit (from 4) | 7.5[1] | ++ |
| 5. | Beef-heart infusion (filtrate from 4) shaken with 50 grams per liter | 0.0 | − |
| 5(*a*). | Acetic-acid extract of norit (from 5) | 0.0 | − |

[1] Average result of several extractions.

The activated adsorbents were extracted with baryta and glacial acetic acid respectively, and the influence of these extracts were tested on the growth of yeast cells and streptococci. The results which we obtained and which are embodied in Tables I and II, show that the substances which stimulate the growth of streptococci and yeast cells, as extracted from beef-heart and autolyzed yeast solutions, apparently belong to the class of vitamines of the water-soluble B type, but are not identical with B vitamine. They are comparable in activity and show similar properties in that they are easily extracted from their natural sources by the same adsorbents, and are again recovered from the adsorbents without appreciable loss in activity.

## ACTION OF PROTEIN HYDROLYSATES ON BACTERIA AND YEAST CELLS.

It is very well known that protein hydrolysates stimulate the growth of certain bacteria, and this stimulating action has been attributed at various times to the presence of unknown substances in the protein molecule. To test out this theory, we subjected to acid hydrolysis twelve animal and ten vegetable proteins, which were prepared and purified by the usual methods, particular care

TABLE III.

QUANTITATIVE ACTION OF PROTEIN HYDROLYSATES ON STREPTOCOCCI.
$p_H$. of Standard Culture Medium = 7.3.

| No. | Hydrolysates of the Proteins. (1 c.c. used in each test.) | Growth. | Change in $p_H$. |
|---|---|---|---|
| 1. | Casein (purified) HCl hydrolysate | + | 5.8 |
| 1(*a*). | " " (sterile control) | − | 7.3 |
| 2. | Casein (purified) $H_2SO_4$ hydrolysate | + | 5.3 |
| 2(*a*). | " " (sterile control) | − | 7.0 |
| 3. | Casein (technical) HCl hydrolysate | + | 5.8 |
| 3(*a*). | " " (sterile control) | − | 7.3 |
| 4. | Gelatin (commercial) | + | 6.0 |
| 4(*a*). | " (sterile control) | − | 7.2 |
| 5. | Gelatin (prepared and purified in laboratory) | − | 7.2 |
| 5(*a*). | " (sterile control) | − | 7.3 |
| 6. | Edestin | + | 6.5 |
| 6(*a*). | " (sterile control) | − | 7.3 |
| 7. | Yeast protein | + | 4.9 |
| 7(*a*). | " " (sterile control) | − | 7.3 |

NOTE.—Hydrolysates of 18 other proteins found to be inactive.

being taken to have them free of vitamines. These hydrolysates were tested on the growth of streptococci and yeast cells. The bacterial growth was measured by the increase in the acidity of the medium, by means of the Sörensen Indicator method. The action on yeast was not constant, and in most cases showed a growth inhibition due to the known inhibiting action of certain amino acids.

The results on streptococci, which are summarized in Table III, strongly suggest that the growth-stimulating action of protein hydrolysates is not due to a constituent part of the protein molecule, but to a vitamine-like substance, probably similar to vitamine D, which is present as an impurity, and which cannot be removed by the known methods of protein purification.

### 93 (1840)

## The existence of different immunological types of B. pertussis.

By CHARLES KRUMWIEDE and LUCY MISHULOW.

[*From the Bureau of Laboratories, Department of Health, New York City.*]

Twenty-two strains having the typical morphological and cultural characteristics of *B. pertussis* have been studied by means of the agglutination and agglutinin absorption reactions. These tests have demonstrated that the cultures studied fall into two serological groups. If the two groups are tentatively designated as "a" and "b" the results may be briefly described as follows: Anti-serums for group "b" agglutinate the strains of group "b" but agglutinate the strains of group "a" very slightly or not at all. The absorption of group "b" serum by group "a" strains does not appreciably reduce the agglutinins for group "b." Group "a" serum, however, agglutinates group "b" strains to a considerable extent. The absorption of group "a" serum by group "b" strains results in a reduction of the agglutinins for strain "a." The serological differences, therefore, are sharply defined in one direction, but group relationship is shown in the reverse direction. These findings are of immediate interest because of their possible bearing on the use of pertussis vaccines.

### 94 (1841)

## The applicability of the precipitin reaction in determining the infectivity of discharges from gonorrheal infections.

By MARGARET F. KELLEY.

[*From the Department of Bacteriology, University and Bellevue Hospital Medical College, New York City.*]

The demonstration of gonococci by either culture or smear method is difficult after the subsidence of acute gonorrheal symptoms. Complement fixation tests on the blood of treated or untreated cases may give negative results. There is need therefore of a method to determine the persistence of infectiousness.

Robinson and Meader[1] reported encouraging results with the application of the precipitin reaction to discharges of gonorrheal origin. We have attempted to verify their results, working under a grant from the U. S. Interdepartmental Social Hygiene Board.

Selected rabbits were immunized with live gonococci to produce the immune serum used for the tests. Specific gonococcus antigen was prepared by autolyzing the gonococcus in salt solution for several days and centrifugalizing to obtain a clear antigen.

"Discharge extracts" from cases were prepared by adding to 2 c.c. of salt solution the secretions obtained from the cervix or vagina. The mixture was allowed to stand over night and then centrifugalized until clear. 0.2 c.c. of clarified extract was then added to 0.2 c.c. of diluted immune serum and to 0.2 c.c. of diluted normal serum as a control. A positive result was shown by the development of a ring of varying thickness and opacity at the point of contact of extract and serum or by the development of a precipitate. The reaction appeared usually from two hours to eighteen hours.

With 92 specimens, smears positive, 82 per cent. gave reactions with gonococcus serum while 21 per cent. gave reactions with normal serum. With 49 specimens, smears negative, 61 per cent. gave reactions with gonococcus serum, while 51 per cent. gave reactions with normal serum. The relatively frequent reactions with normal serum indicated the presence of a non-specific factor and raised the question as to whether the reactions occurring with immune serum alone could be considered specific.

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[1] G. H. Robinson and P. D. Meader, *The Journal of Urology*, 1920, iv, 551.

Of 17 non-gonorrheal vaginal specimens from children 100 per cent. reacted with immune serum and 94 per cent. with normal serum. Nose and throat specimens and a miscellaneous group of sputums, pus due to infection by bacteria other than gonococci and peritoneal washings from normal mice or mice inoculated with exudates due to pneumococcus or streptococcus, gave similar non-specific reactions.

Various methods have been employed in the attempt to eliminate or lessen the non-specific reactions, so that a specific reaction could be recognized if it occurred. The standardization of the opacity of "discharge extracts," the dilution of the serums or of the extracts, or of both, and finally the heating of the extracts have failed to be of help.

With gonococcus serums, precipitates were most frequently encountered with antigens prepared from the staphylococcus and meningococcus. Absorption of gonococcus serums by these heterologous types did not reduce appreciably the reactions obtained with extracts from non-gonorrheal sources.

That the reactions obtained with gonococcus case extracts could not be considered as specific was most conclusively shown by the persistence of reactions after the gonococcus serum was absorbed by the gonococcus. That is, when gonococci were added to the serum to the point where it no longer reacted with a known gonococcus antigen, it still gave a precipitate with extracts from gonococcus cases. Although a specific reaction might have occurred at times, the presence of this non-specific factor would have obscured it.

The precipitin reaction, therefore, as recommended by Robinson and Meader is not applicable for the determination of the presence of the gonococcus in discharges from the cervix, urethra, etc.

### 95 (1842)

## A study of oxalic-acid poisoning.

By **SAMUEL A. BROWN** and **ALEXANDER O. GETTLER.**

[*From the Chemical Laboratory of The University and Bellevue Hospital Medical College and of the Pathological Department, Bellevue Hospital, New York City.*]

The earliest case of oxalic-acid poisoning, reported by Royston, occurred in England, in 1814. Since then, the number of deaths due to oxalic acid and its soluble salts has so increased that today it ranks among the first three poisons in the number of fatalities. A. W. Blyth states that in the five years between 1912 and 1916, there were 448 deaths in England and Wales due to oxalic acid.

The *duration* of a case of oxalic-acid poisoning is usually between 2 and 14 days. There is one case on record by Ogilvie (*Lancet*, 1845) however where death occurred within 3 minutes.

Oxalic acid acts locally as a corrosive and also as a systemic poison. Locally it is more or less destructive to the mucous membrane with which it comes in contact. The lips, tongue, pharynx and esophagus are discolored yellowish white, sometimes marked with patches of a reddish hue. The mucous surface of the stomach is coarsely corrugated and presents a bright red color both in the elevations and depressions; this may change to brown or even black by postmortem action. In some cases the mucous surface is in part or in whole pale, opaque or translucent, and marked by a coarse ramiform vascularity of the submucous tissue. The mucous membrane is soft, pulpy, eroded in patches, thrown into folds, and is easily detached. Perforation is rare.

The systematic effects are attested by falling of the blood pressure, arhythmia and retardation of the pulse, slow breathing, paralytic symptoms and fibrillary muscular contractions. Some consider it a poison acting on the extracardiac ganglia. The red blood corpuscles are destroyed, with consequent fatty degeneration of the tissues. The activity of the muscles is diminished consequent upon loss of irritability. The respiratory muscles are paralyzed.

*Symptoms.*—Although more than 1,000 cases of oxalic-acid poisoning have occurred since Christison wrote his treatise, his description still holds good. "If a person immediately after swallowing a solution of a crystalline salt, which tasted strongly acid, is attacked with burning in the throat, then with a burning in the stomach, vomiting, particularly of bloody matter, imperceptible pulse, and excessive languor, and dies in half an hour, or still more, in twenty, fifteen, or ten minutes, I do not know of any fallacy which can interfere with the conclusion that oxalic acid was the cause of death. No parallel disease begins so abruptly, and terminates so soon; and no other crystalline poison has the same effect." There may also occur headache, cold extremities, numbness and tingling, loss of voice, cramps, convulsions, delirium, coma, etc.

*Prognosis.*—Out of 242 reported cases, there were 132 deaths, a mortality of 54.5 per cent. This ratio, of course, depends upon the manner and speed of treatment. Nor is the outlook for complete recovery favorable if the initial degree of poisoning was severe. There have been a few cases reported in which patients returned after some months suffering from gastric irritability, dyspepsia and symptoms of constriction of the esophagus, the latter due apparently to destruction and subsequent repair of the mucous membrane.

*Postmortem.*—Aside from the local corrosive action, there are no typical pathological lesions with one exception, namely, in the kidneys, where the cortical substance may present a definite whitish appearance due to the presence in the tissues of crystals of calcium oxalate. None is deposited in the glomeruli. Calcium-oxalate crystals have also been found in the blood, bile, aqueous humor, and pleural and pericardial fluids.

*Elimination.*—Is mainly through the urine. The reported analyses show that from 80 to 90 per cent. is excreted through this channel. The urine also contains albumin, a reducing substance and hyaline casts.

*Treatment.*—The oxalic acid should be neutralized and precipitated as quickly as possible, by giving plenty of syrup of lime or a suspension of calcium carbonate. After a few minutes the stomach should be washed out with lime water and, lastly, with plenty of plain water. Stimulants and warmth should be administered to avoid collapse. Diuretics and an abundance of liquid should be given to combat nephritic conditions, and alkalies should be administered to prevent the tendency toward acidosis.

I purpose to present to you to-night a case that came for treatment on the Third Medical Division of Bellevue Hospital, under the direction of Dr. S. A. Brown. This case is of interest because the patient recovered, although antidotal treatment was delayed, and also because of the complete blood study throughout the patient's stay in the hospital.

J. M., a building superintendent, age 44.

*Present History.*—The patient had no bowel movement for 2 days and decided to take what he thought to be a dose of epsom salts. He immediately noticed a peculiar sour taste, with a burning sensation along the esophageal tract, followed presently by severe pains in the epigastrium, which gradually increased. An ambulance was summoned and when the surgeon attempted to examine the patient's throat he vomited. The surgeon, diagnosing it as a case of indigestion, administered sedatives and left. Shortly thereafter the patient developed pain in the lumbar region, the ambulance was summoned again and he was taken to the hospital on October 1, 1921, at 4:30 A.M.

In the course of the succeeding hours he complained of burning pains in the stomach, first localized, then radiating through the abdomen, and of pain and tenderness in the lumbar region. The next morning, after taking a glass of milk, he again vomited.

On physical examination, he was found to be well developed and well nourished. The pupils were equal and regular, reacting readily to light and accommodation. The tongue was clean, the teeth poor, the throat congested. There were no adventitious sounds in the lungs. The heart sounds were of fair quality, no murmurs, rhythm regular, rate 90. The abdomen showed no rigidity, no masses, no tenderness. The spleen was palpable just below the costal margin. The liver edge was also palpable. On deep pressure in the epigastrium, there was pain. There was no edema. Reflexes—no Babinski, no clonus, abdominal cremasteric and knee jerk active.

Treatment consisted of fluid diet, with hot packs, colon irrigations and the following medication: At first bromides and chloral and magnesium sulphate, followed by luminal; later Tr. nux vomica, sodium bicarbonate and veronal. Toward the latter part of his stay in the hospital he was given bismuth subgallate and triple phosphates. It should be noted that no antidotes for oxalic acid were given, as the nature of the poison, if any, was unknown. Upon analysis the salts of which patient had partaken were found to contain 73 per cent. Mg. $SO_4$ and 27 per cent. oxalic acid. The patient stated that he took what is estimated to be 15 to 18 grams of the salt. This means that he obtained 4 to 4.9 grams of oxalic acid. The most common fatal doses are 7 to 15 grams, although there is one case on record in which 3.88 grams proved fatal.

The urine was analyzed for oxalates and found to contain 9.2 mg. of oxalic acid in 100 c.c. of urine. It also contained albumin, white blood cells, red blood cells, hyaline and granular casts.

The blood was analyzed throughout the patient's stay in the hospital, at intervals of a few days, with the following results:

BLOOD ANALYSIS.

| | Time Interval in Days. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 4 | 10 | 14 | 19 | 25 | 28 | 31 | 33 |
| | mg. | mg. | mg. | mg. | mg. | mg. | mg. | mg. |
| Non-protein nitrogen | 85 | 270 | 200 | 60 | 73 | 37 | 40 | 37 |
| Urea nitrogen | 59 | 211 | 149 | 39 | 51 | 16 | 17 | 17 |
| Creatinine | 4.3 | 5.1 | 2.3 | 1.6 | 1.2 | 1.0 | 1.5 | 1.6 |
| Uric acid | 4.1 | 6.3 | 3.2 | 1.5 | 1.0 | — | 1.2 | 1.5 |
| Sugar | 174 | 98 | — | 75 | 70 | — | 85 | — |
| Alkaline reserve, per cent | 48 | 41 | 46 | 55 | — | 59 | — | 55 |

The study of the chemistry of the blood shows a gradual increase in the excretory products. This finding may be explained by the mechanical effects of the calcium-oxalate deposition in the kidneys, seen at autopsy in similar cases. The highest point was reached on the tenth day. In connection with the accepted view that cases in which creatinine is higher than 5 gm. do not recover, it is interesting to notice that the creatinine in this case was 5.1 mg. on the tenth day. A possible explanation for recovery in cases of oxalic-acid poisoning with creatinine over 5 mg. may be that the kidney changes are temporary. The alkaline reserve was below normal throughout the retention period. This evidently indicates that in poisoning by oxalic acid the oxidation processes are subnormal. On the twenty-eighth day all values were back to normal.

The urine output during the first few days was much suppressed; the amount voided per day was 30 to 50 c.c. On about the twelfth day, the urine output suddenly increased to 1,900 to 2,200 c.c. It contained little albumin, few hyaline and granular casts, a reducing substance which did not ferment, together with red blood cells, white blood cells and an increased amount of oxalates.

In view of the fact that over 4 grams of oxalic acid had been taken and that treatment was delayed (in fact, no antidote was given at all), it is interesting to note that recovery occurred. The most plausible explanation for this is the simultaneous taking of magnesium sulphate, which probably hastened the elimination of the poison.

About one month later the patient returned to the hospital complaining of gnawing pain in the epigastric region. This pain usually started a half hour after meals and continued until the next meal. It was aggravated by any kind of food, more so by hot food. He had eructations that were sour in character, was constipated, but not anureous. Fluoroscopic examination showed no ulcer or new growth. The x-ray plates showed a calcareous area in the region of the gall-bladder. This condition is evidently a direct result of the corrosive action of the oxalic acid on the mucous membrane of the stomach.

96 (1843)

## Further studies on ligation of the thyroid arteries in depancreatized diabetic dogs.

By **G. A. FRIEDMAN** and **J. GOTTESMAN.**

[*From the Department of Clinical Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

The experiments described in this and in the following paper were conducted during the year of 1921. 37 dogs were depancreatized; 9 did not develop glycosuria although in some of them repeated pancreatectomies were performed at various intervals. 3 of the animals showed a transitory glycosuria. 25 dogs became persistently glycosuric; 9 of these dogs died of various causes. Ligations of all of the thyroid arteries were done in 8 of 16 glycosuric dogs who survived the pancreatectomies, in about 4 to 7 days after complete or almost complete removal of the pancreas. In one dog the thyroid arteries were ligated in two sittings: first both arteries on the right side and seven days later the arteries on the opposite side. This dog died from tetany on the day following the second ligation. 3 of those who were completely ligated died without demonstrable causes from 1 to 2 days after ligation.

Thus in only 5 dogs could the effect of complete ligation be studied and only in one the effect of partial ligation.

The great losses in body weight which usually follow depancreatizations were not checked by the ligations.

Some interesting points, however, were brought out from the study sufficient to warrant a report. As far as we know there is no literature pertaining to ligations of thyroid vessels in depancreatized dogs.

In a previous paper[1] we referred to an experiment with dog No. 106 who was completely depancreatized. His urine on daily examinations showed from 5 per cent. to 6 per cent. sugar. On the fourth day all thyroid arteries of the animal were ligated. He became sugar-free five days after the operation, and he remained without a trace of sugar in the urine for ten days, when he developed distemper and died from pneumonia.

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[1] Friedman, G. A., and Gottesman, J., PROCEED. SOC. EXP. BIOL. AND MED., 1921, xviii, 281.

*Dog 120.*—Male. Weight 9.6 kilos. Complete pancreatectomy June 22. Glycosuria from June 23 to June 28. Ligation of all thyroid arteries June 28. Sugar positive June 29. Sugar negative on daily examinations of passed and of catheterized specimens from June 30 to July 13. Dog was found dead July 14. Autopsy same day. No demonstrable lesions. Course: good appetite until June 12. No signs of tetany. Sections of the thyroid show many alveoli devoid of colloid (Fig. 1.)

*Dog 108.*—Female. Weight 8.7 kilos. Complete pancreatectomy March 16. Glycosuria from March 17 to March 24. Ligation of all thyroid arteries March 24. Glycosuria persisted until April 1. Died April 2 from emaciation. Autopsy on same day. No demonstrable lesions. Course: poor appetite two days after removal of pancreas. Refused food completely from the day following ligation. No acute attack of tetany, but twitchings of the musculature of the back noted two days following ligation.

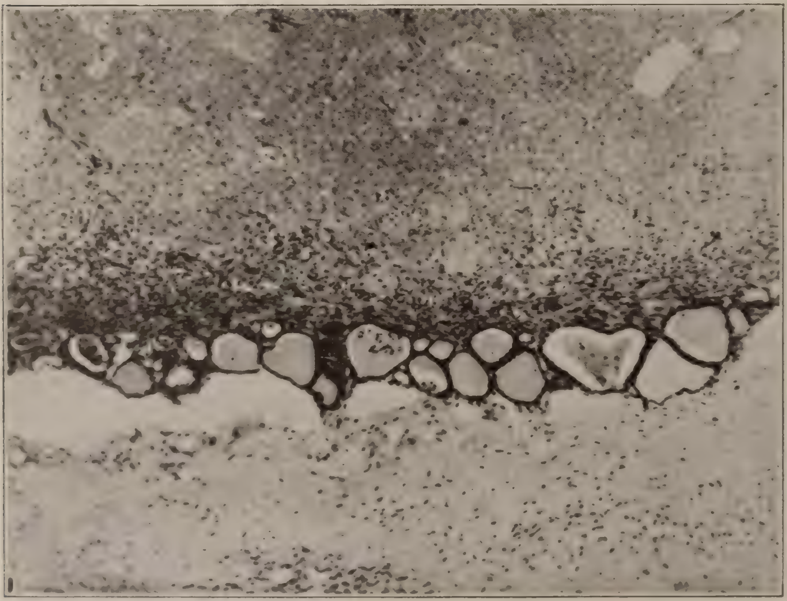

FIG. 1. From dog 123.

Sections of thyroid show extensive hemorrhagic infiltration, marked atrophy. Few remnants of thyroid seen underneath capsule (Fig. 2).

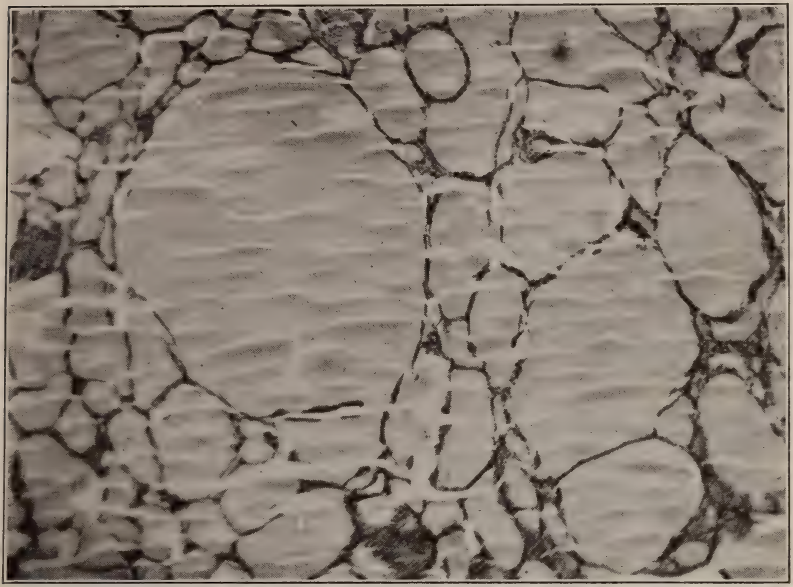

FIG. 2. From normal dog.

*Dog 121.*—Female. Weight 20 kilos. Complete pancreatectomy June 29. Glycosuria from June 30 to July 5. Ligation of all thyroid arteries July 5. Sugar strongly positive July 6. Sugar negative July 7. Sugar positive from July 8 to July 14. Course: slight attack of tetany from July 8 to July 10. July 11 severe attack. July 12 infection noted at neck. Mild attack of tetany. Killed July 14 with chloroform. Large pocket of pus at neck.

*Dog 128.*—Female. Weight 13.8 kilos. Almost complete pancreatectomy July 25. Glycosuria from July 27 to July 30. Ligation of all thyroid arteries July 30. Glycosuria persisted until August 6, when she died. No autopsy. Course: No tetany. Infection at neck noted August 3, four days after ligation.

*Dog 111.*—Male. Weight 6.66 kilos. Almost complete pancreatectomy April 3. Glycosuria from April 4 to April 10. Ligation of superior and inferior arteries on the right side April 10.

## TABLE I.

### BLOODSUGAR IN NORMAL DOGS.

| No. | Mgrm. Sugar per 100 c.c. Blood. | No. | Mgrm. Sugar per 100 c.c. Blood. |
|---|---|---|---|
| 103 | 53 | 110 | 90 |
| 104 | 83 | 111 | 78 |
| 105 | 56 | 121 | 98 |
| 106 | 76 | 122 | 104 |
| 107 | 78 | 123 | 104 |
| 108 | 67 | 124 | 104 |
| 109 | 94 | 133 | 100 |

## TABLE II.

### BLOODSUGAR IN DIABETIC DOGS FOLLOWING PANCREATECTOMY.

| No. | Mgrm. Sugar per 100 c.c. Blood. | No. | Mgrm. Sugar per 100 c.c. Blood. |
|---|---|---|---|
| 104 | 203 | 121 | 238 |
| 105 | 270 | 123 | 127 |
| 106 | 250 | 124 | 100 |
| 107 | 125 | 128 | 228 |
| 108 | 293 | 136 | 250 |
| 109 | 222 | 137 | 200 |
| 111 | 200 | 138 | 290 |
| 120 | 238 | | |

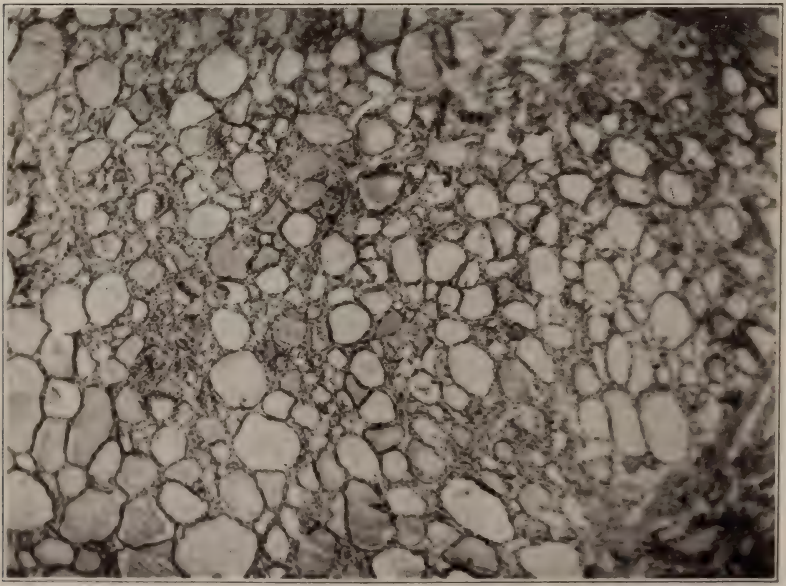

FIG. 3. From dog 120.

Glycosuria from April 11 to April 17. Ligation of superior and inferior arteries on the left side April 17. Sugar positive April 18. Dog died on the following day from a severe attack of tetany. No autopsy. Course: animal was in perfectly good condition until the day following the second ligation. There was a strong ferric-chlorid reaction in his urine on the third day after pancreatectomy. There was no diacetic-acid reaction on succeeding days.

*Table I.*—Bloodsugar estimations were made in these animals before the pancreatectomies. They were starved at least 24 hours preceding the operation. The highest figure obtained 104 mmgr. per 100 c.c. blood in 3 dogs in whom estimations were made during the hot summer months July and August.

*Table II.*—Dog 124 was glycosuric throughout after pancreatectomy, but there was no increase in the contents of bloodsugar. Dog 123 after almost complete pancreatectomy (a minute remnant of pancreas was found at autopsy) did not develop glycosuria while under observation for 26 days. His original weight was 11.2 kilos. On the last day of observation his weight was 9.7 kilos, when he was disposed of with chloroform (loss of approxi-

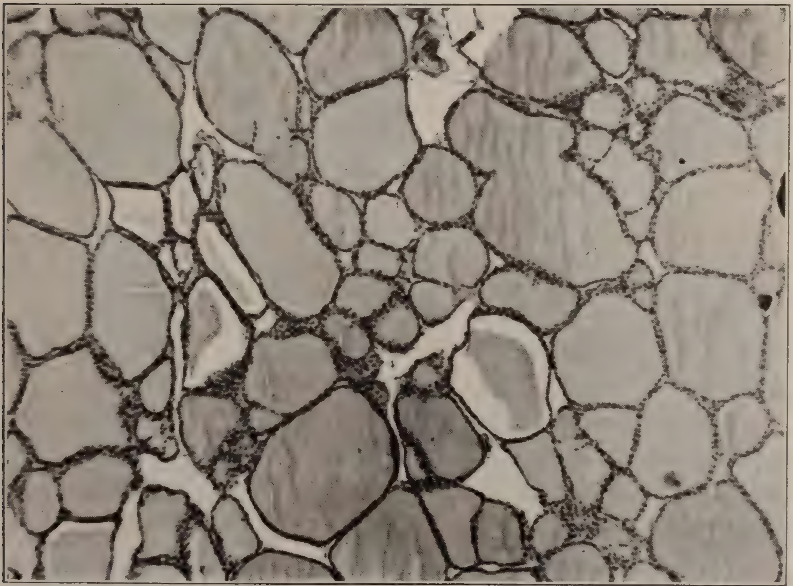

FIG. 4. From dog 108.

mately 60 grm. per day). Such a loss in dogs after almost complete pancreatectomy may be considered as a slight one. Sections of his pancreatic fragment showed very few Langerhans islands and sections from his thyroid (Fig. 3) showed unusually large alveoli rich in colloid. Compare this thyroid with one of a normal dog (Fig. 4) who weighed 13 kilos. We emphasize this point because Cohen[2] and Pariser reported changes in the thyroid in various organic diseases of the pancreas in man whose urine was negative for sugar.

TABLE III.

MMGR. SUGAR PER 100 C.C. BLOOD AND DATES WHEN BLOOD WAS TAKEN FOR ESTIMATION.

| No. | Before Pancreatectomy. | After Pancreatectomy. | After Ligation. | Remarks. |
|---|---|---|---|---|
| 106 | 76 March 2 | 250 March 5 | 192 March 7<br>90 " 10<br>50 " 14 | Animal became sugar-free. |
| 108 | 67 March 16 | 222 March 18<br>293 " 22 | 180 March 25<br>244 " 30 | Bloodsugar diminished after ligation. Increased with tetany. |
| 111 | 78 April 3 | 154 April 7<br>200 " 10 | 244 April 17 | Glycemia increased after partial ligation. |
| 120 | | 238 June 28 | 200 July 1<br>172 " 5 | Animal became sugar-free. |
| 121 | 98 June 29 | 238 July 5 | 213 July 14 | Sugar free 1 day after ligation. Reappearance of sugar with tetany. |
| 128 | | 228 July 30 | | Did not become sugar-free. Tetany absent. Infection present. |

*Table III.*—From this table it becomes evident that partial ligation did not only diminish the diabetic glycemia, but made it more intense.

*Table IV.*—According to Allen[3] the weight of the pancreas in dogs is approximately 2 grm. per kilogram of body weight. This table shows our figures come very close to his in completely depancreatized dogs.

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[2] Cohen, Moritz, and Pariser, Hans, *Dtsch. Med. Woch.*, 1912, $38^1$, 60.

[3] Allen, Frederick M., "Studies Concerning Glycosuria and Diabetes," Boston, W. M. Leonard, publishers, 1913, p. 716.

TABLE IV.

| No. | Body Weight in Kilos. | Weight of Removed Pancreas in Grams. | Weight of Removed Pancreas per 1 Kilo. of Body Weight in Grams. | Approximate Weight of Removed Gland According to Allen. | Remarks. |
|---|---|---|---|---|---|
| 106 | 7.52 | 18.2 | 2.4 | 15.04 | No pancreatic remnant at autopsy. |
| 108 | 8.7 | 21 | 2.4 | 17.4 | Complete pancreatectomy. |
| 111 | 6.56 | 17 | 2.4 | 13.32 | Remnant weight at autopsy 4 grams. |
| 120 | 9.6 | 21 | 2.1 | 19.2 | No remnant at autopsy. |
| 121 | 20 | 44 | 2.2 | 40 | Complete removal. |
| 128 | 13.8 | 20 | 1.45 | 27.6 | Almost complete removal. No autopsy. |

## CONCLUSIONS.

1. The mortality of completely depancreatized dogs after ligation of the thyroid arteries is high.
2. Glycosuria in depancreatized diabetic dogs was checked after complete ligation of the thyroid arteries.
3. Tetany or infection, or both, seem to interfere with the disappearance of the glycosuria.
4. Partial ligation of the thyroid arteries apparently intensifies the diabetes produced by pancreatectomy.

97 (1844)

## The relation of the thyroid and parathyroids to pancreatic diabetes in dogs.

By **G. A. FRIEDMAN** and **J. GOTTESMAN**.

[*From the Department of Clinical Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

Lorand [1] (1904) and McCallum[2] (1909) performed complete thyroidectomies in depancreatized diabetic dogs. The former worked with 3 dogs and the latter with two. In Allen's[1] experiments (1913) with diabetic dogs the thyroidectomies were incomplete. Eppinger,[2] Falta and Rudinger (1908) removed first the thyroid and later the pancreas in two dogs and they did a simultaneous thyroidectomy and pancreatectomy in one dog. Eppinger[3] and his associates (1909) also studied the relation of parathyroid insufficiency to the metabolism in diabetic dogs by removing simultaneously the pancreas with three parathyroids in two animals.

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[1] Lorand, A., *Compt. rend. Soc. Biol.*, 1904, lvi, 488.

[2] McCallum, William George, Johns Hopkins Hosp. Bull., 1909, Sept.

Lorand has asserted that removal of the thyroid sparing the parathyroids is followed by the disappearance of sugar from the urine in two days in depancreatized dogs. In one of McCallum's dogs the glycosuria ceased after removal of the thyroid; in the other one it greatly diminished. In one of the experiments of McCallum two parathyroids were spared; in the other all were left in situ. A marked diminution of sugar after thyroidectomy was also reported by Eppinger. In all these experiments, blood-sugar estimates were not made. The duration of life in the animals of Lorand and McCallum was from one to three days after complete removal of the thyroid even if the parathyroids were left in situ. Three of our diabetic dogs in whom thyroidectomy with partial parathyroidectomy was performed 3 to 4 days after pancreatectomy, died from 1 to 3 days after the operation. From two of these dogs no urine was obtained, and in one dog the glycosuria persisted on the day following the removal of the thyroid.

We may mention here two clinical cases cited by Rohdenburg.[4] One patient was diabetic and later developed exophthalmic goitre. A portion of his thyroid was removed and he remained permanently sugar-free. The other patient had exophthalmic goitre for which a portion of the thyroid was removed. Several years later he developed glycosuria. The glycosuria in this case disappeared after removal of more of the thyroid gland.

We decided that it would be a better procedure to first partially ligate the thyroid arteries and follow this operation on a later date by partial thyroidectomy, or if possible by thyroidectomy alone. One may occasionally succeed in sparing all parathyroids while removing the thyroid especially in larger dogs.

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[1] Allen, Frederick M., 1913, p. 848.

[2] Eppinger, Falta and Rudinger, *Ztschr. f. Klin. Med.*, 1908, lxvi, 1.

[3] Eppinger, Falta and Rudinger, *Ztschr. f. Klin. Med.*, 1909, 380.

[4] Rohdenburg, G. L., "Endocrinology," 1920, iv, 63.

In a previous[1] paper we referred to an experiment with dog No. 100, who became diabetic after removal of a little over one half of his pancreas. Such rare results are occasionally reported in the literature. As the external secretory apparatus of the pancreas was not much affected, but continued functioning, we believe that truly by chance, we produced in this animal a condition which came very close to human diabetes. Inasmuch as this experiment is a singular one in literature we shall briefly refer to it.

Seven days after the dog had from 2 to 3 per cent. sugar in his urine, both inferior thyroid arteries were ligated. The glycosuria persisted on daily examinations. Seven days later both lobes of the thyroid were removed and we succeeded in sparing all of his parathyroids. There was not a trace of sugar in his urine on the day following thyroidectomy and the urine remained sugar-free for 108 days, although eleven days after removal of the thyroid, additional pancreatic tissue was removed, and ninety-three days after the second pancreatectomy the last remnant of the gland was taken out. The animal was sugar-free four days after the third pancreatectomy. He died on the fifth day from prolapse of the intestines, which was probably brought about by the three laparotomies.

The dog's condition was excellent; ate well until the day of the accident. He did not show any signs of myxedema during the time of observation and while his original weight was 14 kilos his weight the day before death was 15.9 kilos, or a gain of 1.9 kilos. His blood sugar remained normal and 14 days after removal of the thyroid his sugar tolerance was about 10 grams per kilogram of body weight. Fig. 1 shows a photograph of the dog 93 days after removal of his thyroid.

Conditions become quite different when one does not succeed in imitating human diabetes and when sparing all the parathyroids in doing a parathyroidectomy is impossible. Infection also changes the situation. It is our impression that diabetic dogs are more susceptible to tetany after thyroidectomy and partial parathyroidectomy than normal dogs. Although in the experiments by one of us[1] in studying the influence of thyroidectomy and partial thyroidectomy on the gastric mucosa, tetany never occurred while leaving in situ 2 or 3 parathyroids; the occurrence of mild or severe attacks after this procedure was the rule in our diabetic dogs. We were able to observe in one diabetic dog a palliative effect on tetany from calcium lactate injections as proposed by McCallum[2] .and Voegtlin with parathyroidectomy in non-diabetic dogs. The following experiments will show the influence of tetany upon the glycosuria in diabetic dogs and the opposite effect of the thyroid.

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[1] Friedman, G. A., and Gottesman, J., PROCEED. SOC. EXP. BIOL. AND MED., 1921, xviii, 281.

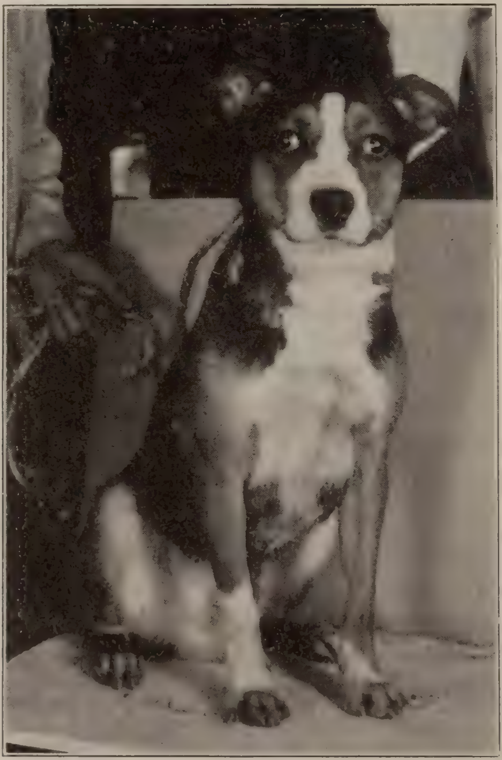

FIG. 1. Dog 100, 93 days after thyroidectomy.

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[1] Friedman, G. A., *Jour. Med. Research*, 1918, xxxviii, 69.

[2] McCallum, W. G., and Voegtlin, Carl, *Jour. Exp. Med.*, 1909, xi, 118.

*Dog 136.*—Male. Weight 13 kilos. Partial pancreatectomy Nov. 9: Glycosuria from Nov. 10 to Nov. 16. Ligation of inferior thyroid arteries Nov. 16. Glycosuria from Nov. 17 to Nov. 30. Thyroidectomy Nov. 30. Superior parathyroids left in situ. Thyroid lobes unusually small. Dec. 1 urine sugar free. Dec. 2 glycosuria. Dog developed a severe attack of tetany early in the morning and died in the afternoon. Autopsy: No demonstrable lesions.

*Dog 138.*—Male. Weight 17.71 kilos. Partial pancreatectomy Dec. 3. Glycosuria from Dec. 5 to Dec. 7. Ligation of inferior thyroid arteries Dec. 7. Glycosuria persisted from Dec. 8 to Dec. 10. Thyroidectomy preceded by intravenous injection of 10 c.c. of 5 per cent. solution calcium lactate. Three parathyroids left in situ. Dec. 12 and 13 sugar positive. Twitchings of the musculature of the back. 50 c.c. calcium chloride 5 per cent. solution by stomach tube daily. Dog developed a severe attack of tetany early in the morning Dec. 14. At 11 A.M. intravenous injection of 10 c.c. calcium-lactate solution 5 per cent. Recovered from the attack; but twitchings persisted. At 2 P.M. second attack of tetany. Dog very low, in a dying condition. At 3 P.M. another injection of calcium lactate, same dosage. Dog was catheterized twice and sugar found in the urine by adding 2 drops of Benedict's reagent. Dec. 15 and 16, no tetany or twitching. Dog had an excellent appetite. Not a trace of sugar in catheterized or passed specimens with either Benedict's or Nylander's reagents. Dec. 17, 18 and 19 twitchings of the musculature of back. No actual attack of tetany. Sugar in urine strongly positive. Mild attack of tetany though the dog had received subcutaneously 10 c.c. of calcium lactate 5 per cent. solution daily. Dec. 20, attack of tetany. Sugar strongly positive in the urine. Intravenous injection of calcium lactate in the morning and in the afternoon. Dec. 21 and 22, no tetany. Urine sugar-free. Subcutaneous injections of calcium lactate were given for three days. Dec. 23 dog was bitten in the back by another dog. On account of the large open wound he was killed.

*Dog 139.*—Female. Weight 12 kilos. Pancreatectomy and ligation of inferior thyroid arteries Dec. 17. Glycosuria from Dec. 18 to Dec. 31. Thyroidectomy Dec. 21. Two superior parathyroids left in situ. Glycosuria persisted from Dec. 23 to Dec. 25; twitchings of musculature of the back were noted the following day. The twitchings persisted though daily subcutaneous injections of calcium lactate were given. Dog was found dead Dec. 26. Autopsy: No pneumonia. Stomach filled with blood. No food present. Mucosa of the pylorus covered with numerous hemorrhagic erosions.

TABLE V.

MMGR. OF SUGAR IN 100 C.C. BLOOD AND DATES WHEN BLOOD WAS TAKEN FOR ESTIMATION.

| No. | After Pancreatectomy. | After Partial Ligation. | After Thyroidectomy. | Remarks. |
|---|---|---|---|---|
| 100 | | | 95 Feb. 15<br>86 " 9<br>66 April 15 | Sugar-free 108 days after thyroidectomy. |
| 136 | 250 Nov. 16 | 285 Nov. 30 | 145 Dec. 2 | Increased bloodsugar after partial ligation. Sugar-free when tetany was absent. |
| 138 | 290 Dec. 7 | 310 Dec. 10 | 238 Dec. 17<br>100 " 22 | Increased bloodsugar after partial ligation. Glycosuria absent on days when tetany free. |
| 139 | 228 Dec. 21 | 228 Dec. 21 | | Pancreatectomy and partial ligation in one sitting. Did not become sugar-free. Mild attacks tetany after removal of thyroid. |

*Table V.*—Note the increase of bloodsugar in dogs 136 and 138 after partial ligation. A similar increase in dog 111, previously reported, after the same procedure. In three dogs the diabetes caused by pancreatectomy was not checked by partial ligation, but became even more intense. Compare these figures for bloodsugar with the normal figures in dog 100 after thyroidectomy with the diminished amounts after thyroidectomy with partial parathyroidectomy in the other dogs. The bloodsugar of dog 138 which amounted to 100 mgr. was found on a day when he was free of urinary sugar and free from tetany. We did not succeed in obtaining blood from dog 139 after thyroidectomy as his veins at the neck collapsed so that it was impossible to introduce a needle.

TABLE VI.

| No. | Body Weight in Kilos. | Weight of Removed Pancreas in Grams. | Weight of Removed Pancreas per 1 Kilo of Body Weight. | Approximate Weight of Gland According to Allen. |
|---|---|---|---|---|
| 100 | 14 | ?<br>3.4<br>10 | ? | 28 |
| 136 | 13 | 17 | 1.3 | 26 |
| 138 | 15.71 | 26 | 1.65 | 31.42 |
| 139 | 12 | 23 | 1.91 | 24 |

Table VI indicates that it is possible to obtain a persistent glycosuria after removal of 1 to 1.3 grams of pancreas per 1 kilogram of body weight. Allen[1] figures that the pancreas of a dog weighs approximately 2 grm. per kilogram of body weight. This makes us believe that we probably removed 14.6 grams in dog No. 100 at the first operation.

## CONCLUSIONS.

1. Diabetic dogs are more susceptible to tetany after partial parathyroidectomy and thyroidectomy than non-diabetic dogs after the same procedure.
2. The removal of the thyroid in diabetic dogs seems to check the glycosuria provided tetany does not occur.
3. If tetany does occur intravenous injections of calcium lactate may act as a palliative in checking temporarily both the tetanic seizures and glycosuria.

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[10] Allen, l.c., 716.

### 98 (1845)

## Concerning the amount and distribution of stainable lipoid material in renal epithelium in normal and acutely nephropathic animals, with observations on the functional response of the kidney.

By **WM. DEB. MACNIDER.**

[*From the Laboratory of Pharmacology of the University of North Carolina, Chapel Hill, North Carolina.*]

The following observations are based upon a study of twenty-six dogs. The animals were under two years old. The dogs were placed in metabolism cages and studied for eight days prior to any experimental interference. During this period, as well as during the period of the experiments, the animals were given 250 c.c. of water twice a day by stomach tube. The diet consisted of scraps of bread and cooked meat. The urine was collected once a day and examined qualitatively for albumin and glucose. The phenolsulphonephthalein test for renal function was made every other day according to the technique of Rowntree and Geraghty. Daily determinations of the alkali reserve of the blood (R.p.H.) were made by the method of Marriott. Blood-urea determinations were made by the method of Marshall as modified by Van Slyke and Cullen.

At the end of the eight-day period allowed for normal observations, seven of the animals were killed without the use of an anesthetic and used as control experiments. The remaining nineteen animals were given one subcutaneous injection of 6 mgs. of uranium nitrate per kilogram. Following the use of uranium, observations similar to those previously outlined were continued. The animals that were given uranium were killed without the use of an anesthetic 6 hours, 12 hours, 24 hours and 48 hours following the commencement of the intoxication. At the termination of the observations on both the normal control animals and the acutely nephropathic animals, kidney tissue was at once obtained for microscopic study. Sections of tissue extending through each lateral half of both kidneys were placed in isotonic salt solution and without any fixation were frozen, sections made, and stained for lipoid material by Herxheimer's Scharlach R. method. Such sections were counterstained with Mayer's Haemalum. Other tissue from both kidneys was fixed with formaline, Zenker's fluid, and in corrosive-acetic, imbedded in either paraffin or celloidin, and used for a general histological study.

## NORMAL CONTROL ANIMALS.

During the eight days of observation, urine formation by the seven normal control animals has varied from a minimum output of 385 c.c. to a maximum output of 621 c.c. The urine was free from both albumin and glucose. The elimination of phenolsulphonephthalein by the respective animals in a two-hour period varied from 65 per cent. to 80 per cent. The blood urea varied from 12 to 18 mgs. per 100 c.c. of blood. The reserve alkali of the blood was normal and gave readings between 8.0 to 8.1.

When such animals were killed without the use of an anesthetic and kidney tissue studied for the amount and distribution of stainable lipoid material by the use of Scharlach R., the following observations were made. The endothelium of the glomerular capillaries and other vascular tissue of the kidney failed to show the presence of stainable lipoid. The convoluted tubule epithelium in young animals such as have been used in this study does not show the presence of stainable lipoid with Scharlach R. In old normal animals as has been previously noted,[1] stainable lipoid may appear in the epithelium of this portion of the tubule in the form of fine dust-like particles. All of the normal control animals show stainable lipoid in both the descending and ascending limbs of Henle's loops. In this portion of the tubule such material appears as small particles or fused droplets.

The study of the normal control group of animals indicates that stainable lipoid as demonstrated by Scharlach R. with Herxheimer's technique of staining is confined to the epithelial cells of the loops of Henle. Lipoid material in this location has no harmful effect on the functional capacity of the kidney and does not interfere with that function of the kidney which is concerned with maintaining a normal acid-base equilibrium of the blood.

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[1] MacNider, Wm. deB., *Jour. Pharm. and Exp. Therap.*, 1921, xvii, 289.

## ACUTELY NEPHROPATHIC ANIMALS.

The experiments on the animals acutely nephropathic from uranium were terminated as follows. Four animals were killed six hours after the administration of uranium, five at the end of twelve hours, five at the end of twenty-four hours and the remaining five animals at the end of forty-eight hours.

The four animals killed six hours following the administration of uranium showed no change in the normal functional response of the kidney. During this period the animal of Experiment 4 formed 129 c.c. of urine. The urine was free from both albumin and glucose. Casts were not present. The elimination of phenolsulphonephthalein during the last two hours of the intoxication was 75 per cent. as compared with the output of 72 per cent. of the dye prior to the use of uranium. The blood urea remained unchanged from the normal reading of 18 mg. per 100 c.c. of blood. The reserve alkali of the blood was unaffected and remained at the normal reading of 8.1.

A study of the kidneys of the four animals killed at this early period of the intoxication shows the glomeruli to be normal. Stainable lipoid is not present in the endothelium of the capillaries. The stainable lipoid in the cells of the loops of Henle, especially in the cells of the ascending limb of the loop, shows an increase in amount when compared with the amount of such material that can be demonstrated in this location in the normal control animals. The lipoid is in the form of granules and well-defined droplets. The convoluted tubule epithelium failed to show stainable lipoid with Scharlach R. at this early stage of the intoxication. These cells appear normal.

The five animals killed at the end of twelve hours following the use of uranium have all shown some change from the normal in the functional response of the kidney. In the urine from three of the animals both albumin and glucose were present. The urine of the two remaining animals contained a trace of glucose but no albumin. The results obtained in Experiment 7 are characteristic for this group. During the twelve-hour period of the experiment the animal formed 322 c.c. of urine. Heavy traces of both albumin and glucose were present. The elimination of phenolsulphonephthalein was reduced from the normal of 70 per cent. to 55 per cent. Blood urea was unchanged. The reserve alkali of the blood was reduced from 8.05 to 7.95.

Frozen sections from the kidneys of this group of animals when stained with Scharlach R. show an increase in the amount of stainable lipoid in the cells of the loops of Henle and, furthermore, at this stage of the uranium intoxication, stainable lipoid in the form of dust-like particles appears in the convoluted tubule epithelium. The granules are more marked in the periphery of the cells than in the area immediately around the nuclei. Other than these changes in the stainable lipoid content of the tubular epithelium the kidney tissue appears normal. A study of the course of the intoxication to this point shows the first evidence of an injury to the kidney from uranium to consist of inducing such a disturbance in the cells of the loops of Henle that an increase over the normal of stainable lipoid can be demonstrated in these cells. Such a change in the stainable lipoid content of these cells is not associated with any functional disturbance on the part of the kidney. At a later period in the intoxication, after twelve hours, lipoid material stainable with Scharlach R. appears in the convoluted tubule epithelium. With this evidence of injury to these cells the elimination of phenolsulphonephhtalein is reduced; there is a beginning depletion in the alkali reserve of the blood, and albumin and glucose or glucose alone appear in the urine. The total output of urine in such animals is apparently unaffected.

Five animals were killed at the end of the twenty-four-hour period of the intoxication. The formation of urine at this stage of the experiments shows an increase over the normal daily output for the respective animals. The urine from all of the animals shows albumin and glucose. Granular casts are present. The elimination of phenolsulphonephthalein is further reduced. There is no retention of blood urea. The reserve alkali of the blood was depleted in all of the animals.

Experiment 10 is representative of the group. The average daily output of urine for this animal before the commencement of the intoxication was 410 c.c. The urine increased to 618 c.c. on the first day following the use of uranium. The urine contained 1.8 gm. of albumin per liter and 1.1 per cent. glucose. The elimination of phenolsulphonephthalein was reduced from the normal of 75 per cent. to 30 per cent. There was no retention of blood urea. The reserve alkali of the blood was reduced from 8.05 to 7.9.

Frozen sections from the kidneys of the animals at this stage of the intoxication after staining with Scharlach R. show very little change in the amount of stainable lipoid in the cells of the loops of Henle. Such material is abundant and in the form of droplets and fused masses. There is a marked increase in the amount of stainable lipoid in the cells of the convoluted tubules. In this portion of the tubule the small particles that have been described as appearing in this location at an earlier period of the intoxication have fused so as to form small droplets which are numerous. In addition to this change in the convoluted tubule epithelium, these cells show marked cloudy swelling and a commencing vacuolation. The capillaries of the glomeruli are engorged with blood. They fail to show the presence of stainable lipoid or other evidence of injury.

The remaining five animals were killed at the end of forty-eight hours of the intoxication. In two of the animals the formation of urine was in excess of the normal daily output. In three of the animals there was a reduction in urine formation. Urine from all of the animals has shown an increase in albumin and glucose over that observed at the end of twenty-four hours of the intoxication. The elimination of phenosulphonephthalein has shown a progressive decrease. Only two of the animals show a retention of blood urea. The reserve alkali of the blood shows a progessive depletion. The results obtained in Experiment 16 are representative of the group. The formation of urine was reduced from the average normal daily output of 421 c.c. to 248 c.c. The urine contained 4.7 gm. of albumin per liter and 2.08 per cent. glucose. The elimination of phenolsulphonephthalein was reduced from the normal output of 68 per cent. to 10 per cent. Blood urea had increased from 14 to 42 mg. per 100 c.c. of blood. The reserve alkali of the blood was reduced from 8.1 to 7.85.

Frozen sections from the kidneys of these animals when stained with Scharlach R. show little if any increase in the amount of stainable lipoid material in the cells of the loops of Henle or in the convoluted tubule epithelium. Other changes of degeneration in these cells that have been preceded by the appearance of stainable lipoid have become more marked. The cells show an advanced swelling, which frequently obliterates the lumen of the tubules. Vacuolation and necrosis are well advanced in many of the cells, especially in those of the convoluted tubules. The glomerular vessels are engorged with blood. Occasionally a slight exudate is seen in the subcapsular space. The endothelium of the capillaries has failed to show stainable lipoid.

## Conclusions.

1. Lipoid material stainable with Scharlach R. is constantly found in the cells of the loops of Henle in normal dogs. The presence of such material in this location is not indicative of a pathological kidney. The functional capacity of such a kidney is normal.

2. When animals are given one subcutaneous injection of 6 mg. of uranium nitrate per kilogram, the earliest evidence of injury to the kidney consists of an increase in the amount of stainable lipoid in the cells of the loops of Henle. At this stage of the intoxication there is no evidence of a functional disturbance on the part of the kidney and no change takes place in the acid-base equilibrium of the blood.

3. At a later stage of such an intoxication (12 hours) stainable lipoid material appears in the convoluted tubule epithelium. The vascular tissue of the kidney is uninjured. Associated with such a disturbance in the metabolism of these cells that leads to the appearance of stainable lipoid in the cell there occurs a reduction in the elimination of phenolsulphonephthalein, a depletion in the alkali reserve of the blood and the appearance of albumin and glucose, or glucose alone in the urine.

4. Following this initial injury to the tubular epithelium changes of a more distinctly degenerative type appear in these cells and the functional capacity of the kidney is more severely impaired.

99 (1846)

## Preliminary report on the effects of vagus stimulation on the dog's stomach and the influence of asphyxia on these effects.

By **Z. BERCOVITZ.**

*[From the Hull Physiological Laboratory of the University of Chicago and Physiological Laboratory of Baylor Medical College, Dallas, Texas.]*

In a previous report[1] attention was called to the fact that in the turtle repeated vagus stimulation was followed by a progressive decrease in the gastric response to each stimulation. It was further pointed out in another report[2] that the stomach could not be tetanized by prolonged vagus stimulation, also that reduction in the temperature of the turtle was followed by a decreased gastric response to vagus stimulation. These facts seemed to indicate that in the turtle a complex neuro-muscular mechanism controlled the response of the stomach to vagus stimulation.

The object of this study therefore was to determine if the dog's stomach would respond to vagus stimulation in the same manner as the turtle's stomach.

### METHODS.

The dogs used in this study were decerebrated in order to avoid the depressing influence of an anesthetic.

The balloon method was used for recording gastric contractions. The balloon was introduced into the stomach through (*a*) healed gastric fistula; (*b*) through slit in the anterior wall of the stomach near the pylorus; (*c*) through the mouth and esophagus; (*d*) through the duodenum. The results were the same in all cases.

In most cases a simultaneous blood-pressure tracing was made from the carotid artery and in others the chest was opened, artificial respiration given and the heart observed directly.

To produce asphyxia of the stomach a lifting ligature was placed under the thoracic aorta.

The animals were cooled by packing them in cracked ice until the rectal temperature dropped to the desired point. As a rule 1½ to 2½ hours were required to cool the decerebrated dog to 23° C.

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[1] Bercovitz and Rogers, *Amer. Journ. Physiol.*, 1921, lv, 323.

[2] Rogers and Bercovitz, *Amer. Journ. Physiol.*, 1921, lvi, 257.

## RESULTS.

*Effect of Repeated Vagus Stimulation.*—In the dog stimulations of the vagus with a tetanizing current repeated at short intervals with a given strength of current are followed by contractions of the stomach of approximately uniform amplitude. Complete cardiac inhibition was observed during each stimulation. The apparent ability to produce an artificial rhythm of the dog's stomach by repeated stimulations of the vagus is in striking contrast to the rapid failure of gastric contractions on repeated vagus stimulation in the turtle.

*Effect of Prolonged Vagus Stimulation.*—Prolonged stimulation of the vagus with a tetanizing current of moderate strength and also a strong tetanizing current is followed by only a single contraction of the stomach and its usual subsequent relaxation in spite of continued stimulation. Weak peristalses in the pyloric region may occur after the one contraction during the balance of the stimulation but usually stops shortly after the stimulation has ceased. There is no indication of a tetanus or increased tone of the stomach. These results are similar to those obtained in the turtle.

It was noted from the blood-pressure tracings and also from direct observation of the heart that complete vagus inhibition of the heart failed at about the same time as the relaxation of the stomach began. It would seem that the mechanism operating to prevent gastric tetany is the same as that which prevents prolonged complete cardiac inhibition from vagus stimulation.

*Influence of Cooling.*—In dogs cooled to 25° C. to 23° C. it was found that repeated stimulations of the vagus were followed by no indications of either failure of gastric response or increased or permanent tone as the result of the stimulations. Prolonged vagus stimulation at this temperature is not followed by tetanus of the stomach.

It was further noted that at a temperature of 21° C. there was a failure to obtain a gastric response from vagus stimulation but stimulation of the stomach wall was followed by a contraction. No cardiac inhibition was noted at this temperature in response to vagus stimulation. As the temperature was raised there was a corresponding increase in gastric response to stimulation of the vagus.

The heart seemed to be more easily thrown into fibrillation in the animals which had been cooled than in correspondingly prepared animals at normal body temperature. Death in most of the experiments on cooling was due to fibrillation of the heart.

*Influence of Temporary Asphyxia.*—In dogs with the thoracic aorta ligated repeated stimulations of the vagus are followed by rapid failure of gastric response to stimulation. Allowing the blood to pass again to the stomach is followed by recovery of the gastric response to vagus stimulation.

These experiments would seem to indicate that in the dog as well as in the turtle a complex neuro-muscular mechanism influences the effects of vagus stimulation on the stomach. This mechanism in the dog is very sensitive to partial asphyxia. It may be in the turtle that the rapid failure of gastric response to vagus stimulation is dependent on circulatory changes causing an asphyxia of the complex neuro-muscular mechanism.

## SUMMARY.

1. In the dog stimulations of the vagus repeated at short intervals are followed by contractions of the stomach of approximately uniform amplitude.

2. Prolonged stimulation of the vagus is followed by a single contraction of the stomach and its usual subsequent relaxation in spite of continued stimulation. Failure of cardiac inhibition occurs at about the same time as relaxation of the stomach begins.

3. Reduction of the body temperature to 25° C. to 23° C. is followed by decreased response to vagus stimulation but no indications of either failure of gastric response to repeated vagus stimulation or tetanus. At 21° C. vagus stimulation is without effect on the stomach but stimulation of the stomach wall is followed by a contraction.

4. Partial asphyxia of the stomach by shutting off the blood supply to the part is followed by rapid failure of gastric response to repeated vagus stimulations similar to that observed in the turtle. Removal of the asphyxia is followed by recovery of the gastric response to stimulation.

### 100 (1847)

## Agglutination phenomena with diphtheria antitoxin.

By **P. J. MOLONEY** and **L. O. HANNA.**

[*From the Research Division, Connaught Antitoxin Laboratories, University of Toronto, Toronto, Canada.*]

In search for an in vitro test for diphtheria antitoxin the following observation was made: (*a*) When an emulsion of the diphtheria bacillus, Park 8, is mixed with diphtheria antitoxin, allowed to stand at 37° C. for 1 hour, centrifuged, washed with saline and re-suspended, it is no longer agglutinated by diphtheria-agglutinating serum. (*b*) The organisms sensitized in this way are inagglutinable by acid agglutination.

In this test for the inhibition of acid agglutination the cells are suspended in a buffer solution which gives a maximum agglutination with unsensitized cells. The point of maximum agglutination for acids varies somewhat depending on the culture and the buffer mixture, but for phthalate mixtures (Clark and Lubs) diluted 1–1 with distilled water it is about $P_H$ 4.2 for a three or four-day broth culture of the Park 8 strain.

To determine the specificity of the test for diphtheria antitoxin, experiments were carried out along four different lines.

1. The following sera were used in place of diphtheria antitoxin and the test carried through: normal human serum, positive T.B. human serum, normal guinea pig, concentrated tetanus antitoxin, fresh antitoxic serum, normal horse, normal sheep, normal rabbit.

In the above experiment there was inhibition of agglutination when the cells had been sensitized with antitoxic serum and with tetanus antitoxin; and when the other sera had been used there was agglutination. A guinea-pig test with the tetanus antitoxin showed that it contained diphtheria antitoxin, and it was subsequently discovered that this tetanus antitoxin was from a horse which had previously been used for the production of diphtheria antitoxin.

When diphtheria bacilli are sensitized with diphtheria agglutinating serum instead of antitoxin there is inhibition of agglutination when the cells are subsequently subjected to serum agglutination but acid agglutination is not inhibited.

A possibility which was kept in mind was that the effect with antitoxin might be due to the presence of agglutinoids in antitoxic serum. In an attempt to check this, agglutinating serum diluted (1–10) was heated 75–80° C. for 1 hour and subsequently used in the test; both with acid and serum agglutination there was no inhibition. One might conclude from this experiment that the agglutinins were destroyed by the high temperature and that no agglutinoids were produced. Up to the present attempts to produce agglutinoids from agglutinins have not been successful.

2. An emulsion of diphtheria organisms incubated with a mixture of antitoxic serum and diphtheria toxin in suitable quantities is subsequently agglutinated both by acid and by diphtheria-agglutinating serum, whereas if the toxin is replaced by an equal volume of broth the cells are not agglutinated. The conditions for this experiment are limited by two factors: there must be sufficient antitoxic serum to sensitize the cells, and an excess of toxin.

Diphtheria organisms sensitized with antitoxin are rendered agglutinable by mixing with diphtheria toxin, centrifuging, washing with saline and re-suspending.

Diphtheria toxin has this same neutralizing effect on diptheria-agglutinating serum. From this one might conclude that diphtheria toxin contains agglutinogens besides true toxin.

3. In an attempt to apply the test quantitatively two general methods were used: Organisms were sensitized with progressive dilutions of antitoxic sera and the limit of inhibition read as the end point; and mixtures of serum and toxin after standing 1 hour at room temperature were added to sensitized cells directly, and after dialyzing—it had been found that toxin dialyzes through parchment paper—and the dialysate mixed with sensitized cells which were subsequently tested for agglutinability.

Neither method gave results which were uniformly consistent with guinea-pig experiments. Using the first method some results were obtained which were parallel to the guinea-pig tests, in other cases the results were reversed, *i.e.*, in some cases two sera which by Ehrlich's method gave, *e.g.*, 300 Å.U. and 150 Å.U. per c.c.

respectively showed by this test that the second contained more antitoxin than the first.

In this connection it is well to recall that according to the work of Roux,[1] Danysz,[1] Momont[1] and Cruveilhier,[2] the results obtained with antitoxic sera by Ehrlich's method do not always parallel those obtained by the French method—a method which has at least the merit that its results are based on animal experiments, the conditions of which correspond in a measure to those which obtain in the actual treatment of the disease. These workers claim that in some cases the results are not only not parallel but may even be the reverse of each other. On the other hand according to the work of Marx[3] the two methods give parallel results. No comparison between the test described here and the French method has yet been carried out.

4. It would be of interest to know whether washed diphtheria organisms do actually take up antitoxin. A carefully controlled experiment to test this was carried out. Organisms which had been washed several times with saline were mixed with a known amount of antitoxin, allowed to stand for 1 hour at room temperature, centrifuged and the supernatant liquid drawn off. A guinea-pig test with an appropriate amount of toxin showed a fall in antitoxic content of the mixture. The difficulty with this experiment is that it is not known when the cells are sufficiently washed. It is proposed to repeat this experiment in the following way: Wash diphtheria cells several times and then wash again batches of these cells and test the first and progressively washed lots to see whether a point is reached where the drop in antitoxin reaches a constant.

## SUMMARY.

Both acid and serum agglutination of Park 8 strain of *B. diphtheriæ* are inhibited when the organisms are first sensitized with diphtheria antitoxin; when diphtheria-agglutinating serum is used instead of antitoxin, serum agglutination is inhibited but not acid agglutination.

This inhibition phenomenon with antitoxin is specific, at least for the Park 8 strain.

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[1] Abstract in the "Bacteriology of Diphtheria"; Nuttall, Graham, Smith, 1913, 525.

[2] Cruveilhier, *Ann. Inst. Pasteur*, xix, 1905, 249.

[3] Marx, *Zeit. f. Hygiene*, 1901, xxxviii, 372.

It is not definitely established whether it is a test for antitoxin; it is possible that the phenomenon may be due to agglutinoids, or to a diphtheria antibody not noted heretofore.

Nicolle, Debains and Césari[4] have described a qualitative test for toxin and antitoxin which is based on a precipitin reaction. It is evident from the results above that while the test may be specific for the diphtheria bacillus and for the other organisms used by them, it should be subjected to further investigation before it can be accepted as specific for toxin and antitoxin as defined by Ehrlich's guinea-pig test.

### 101 (1848)

## Action of some purin derivatives on the isolated bronchus.

By DAVID I. MACHT and GUI CHING TING.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*]

In connection with a study of the effects of various drugs on the isolated bronchi of pigs the authors studied a number of purin derivatives. The effects of caffein or trimethyl xanthin in doses of 1–20 mgm. in 25 c.c. of Locke's solution gave the following results; small doses produced no effect on bronchial muscle or occasionally a very slight constriction. After large doses of caffein a little relaxation of the normal bronchial preparations was noted. When, however, such bronchial preparations were first brought into a state of high tonus or contraction, as for instance on treatment with muscarin, the relaxing effect of a subsequent dose of caffein was much more marked. On the whole, however, the results obtained indicated that caffein has a very weak dilator effect on the bronchus.

Following experiments with caffein, observations were made on the effects of theobromin or 1–3 dimethyl-xanthin and theocin or 3–7 dimethyl-xanthin. It was found that both dimethyl-xanthins produced much greater broncho-dilatation than trimethyl-xanthin or caffein. The authors were unable to obtain a mono-methyl xanthin but they did study the effects of xanthin itself. Although

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[1] Nicolle, Debains and Césari, *Comp. rend.*, 1919, clxix, 1433.

xanthin is very slightly soluble nevertheless even very minute quantities of the substance (1 c.c. of 1–200,000 solution) introduced into 25 c.c. of Locke's solutions in which the preparation was suspended were found to produce a very marked relaxation. Hypoxanthin acted in the same way. Going a step further experiments were made with minute quantities of guanidin and adenin and both of these were found to produce relaxation of the bronchus and seemed to be comparatively more potent even than xanthin. Passing to the nucleosid guanosin, the pharmacological action became different. Guanosin produced no effect. A few experiments with adenin nucleotid showed that it also was inactive. Finally tests made with solutions of thymus nucleic acid and yeast nucleic acid gave also no effect on the bronchial muscle.

### 102 (1849)

## Effect of cocaine on the growth of lupinus alba: a contribution to "phytopharmacology."

By **DAVID I. MACHT** and **MARGUERITE LIVINGSTON**

[*From the Pharmacological and Plant Physiology Laboratories, Johns Hopkins University, Baltimore, Md.*]

The effects of cocaine and its decomposition products were studied on the growth of seedlings of the plant *Lupinus alba*. The seeds were soaked in water and allowed to sprout in a suitable medium, following which the length of the straight roots grown by this plant was measured and the effects of cocaine and other chemicals on the growth of the roots were investigated. The plants were placed in solutions of nutrient salts (Shive solution) and the various drugs were added to such solutions. Controls were made on seedlings suspended in Shive solution diluted one half with distilled water. It was found that the effect of cocaine and its decomposition products on the growth of lupinus was very different from the effect of the same substances on animal tissues. Whereas cocaine is very toxic for animal tissues such as smooth and skeletal muscle, nerves, etc., it required strong solutions of this alkaloid, namely 2 per cent. of cocaine hydrochloride to inhibit the growth of the seedlings. Ecgonin hydrochloride inhibited growth in concentrations of .0055 per cent., while benzoyl ecgonin was much less toxic, requiring 2½ per cent. concentration to affect the growth. Methyl alcohol was found to be very little toxic to the roots of the lupinus, requiring 4.8 per cent. to produce an inhibition of growth. Contrary to expectation the most toxic decomposition product of cocaine was found to be sodium benzoate, a compound which is practically non-toxic for animal tissues. Sodium benzoate was found to be deleterious to the lupinus root in concentrations of 0.007 per cent.; while the ester methyl-benzoate was found to produce inhibition in concentrations of 0.014 per cent. Various simple mixtures of ecgonin, sodium benzoate, methyl alcohol, benzoyl ecgonin, etc., were also studied and the effects of these will be described in the complete paper in the Journal of General Physiology.

### 103 (1850)

## Spontaneous cure of rickets in rats.

By **ALFRED F. HESS, LESTER J. UNGER** and **A. M. PAPPENHEIMER.**

[*From the Department of Pathology, College of Physicians and Surgeons, New York City.*]

It has long been believed that in infants healing of rickets occurs in spite of the diet and environment remaining unchanged. Some time ago in the course of an experiment on the curative effect of sunlight in the rickets of rats, it was observed that rickets healed in two of the control animals. One of these rats was on the Sherman and Pappenheimer diet plus 25 mg. P. per cent. and the other on the same diet with an addition of 50 mg. P. per cent. The rats weighed 34 g. at the outset, and after 30 days showed rickets by x-ray. After 62 days the radiograph was negative in one instance and demonstrated healing in the other. Autopsies of both revealed no gross rachitic changes; microscopic examination showed healed rickets at the costo-chondral junctions in one instance, in the other no rickets was found but some autolysis had taken place.

| Rat | Diet | Wgt. | X-Ray 18 ds. | Wgt. | X-Ray 32 ds. | Wgt. | X-Ray 48 ds. | Wgt. | X-Ray 74 ds. | Wgt. | Pathology. | | Inorg. P. mg. %. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | | Gross. | Micros. | |
| 631... | 84 + 25 mg. P. per cent. | 36 | Slight R. | 54 | Mod. R. | 62 | Healed R. | 58 | Healed | 50 | No. R. | Osteoporosis following previous R. | 3.46 |
| 633... | do. | 40 | R. | 54 | Mod. R. | 60 | Healing R. | 60 | Healed | 50 | No R. | Inactive Osteogenesis<br>No R. | 3.0 |
| 635... | do. | 38 | Slight R. | 50 | Slight R. | 54 | Healing R. | 64 | Healed | 60 | No R. | Healing R. | 3.75 |
| 639*A* . | do. | 40 | Almost neg. | 64 | Mod. R. | 70 | Healing R. | 68 | Healed | 54 | No R. | Osteoporosis | |
| 445... | do. | 34 | | | Rickets | | Marked R. | | Healing R. | 50 | No R. | No R. (Autolysis) | |

In view of this experiment another series of rats were put on the same diet (Sherman and Pappenheimer plus 25 mg. P. per cent., in the form of secondary potassium phosphate). This addition furnishes a level of phosphorus which is still inadequate for the rat, and leads to rickets. Of the four receiving this diet all showed signs of rickets according to x-ray after 32 days. After 48 days, however, the radiographs showed healing. During this period the rats had made a total gain of 92 g. After 74 days the x-ray showed the lesions apparently healed, and the rats were killed. No gross evidences of rickets were found at necropsy. Microscopic examination of the costo-chondral junctions also showed no evidences of active rickets; the rickets was either healing or there was merely osteoporosis.

The explanation suggested for this spontaneous healing is that, with a practically stationary weight over a long period, the phosphorus requirement for the building up of new tissue is greatly reduced. The small addition of P. under such circumstances, to the standard rickets-producing diet, suffices to enable the bone to recalcify.

### 104 (1851)

## A further report on the prevention of rickets in rats by light rays.

By ALFRED F. HESS, LESTER J. UNGER, and A. M. PAPPENHEIMER.

[*From the Department of Pathology, College of Physicians and Surgeons, New York City.*]

In a previous communication we showed that the development of rickets in rats fed the standard rickets-producing diet of Sherman and Pappenheimer can be prevented by daily exposures to direct sunlight for fifteen minutes.[1] A similar result has been reported by others.[2] If the rats are placed in a box having flint glass windows, it was found that the sun's rays which had traversed the glass had lost their protective power. Rays which were reflected to the rats from a white surface retained some of their efficacy. Rickets can be prevented in rats by daily exposures of about 2 minutes to the rays of the mercury vapor quartz lamp, or by 4 minutes or less exposure, at a distance of three feet, to the rays from a carbon arc lamp. X-rays were found ineffective.

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[1] Hess, A. F., Unger, L. J., Pappenheimer, A. W., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 8.

[2] Shipley, P. G., Park, E. A., Powers, G. F., McCollum, E. V., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 43.

In order to test the effect of the pigment of the skin on the protective action of light, a group of white and another group of black rats (the melanic form of the Norway rat) were exposed to the radiation from the mercury lamp. In the first experiment both sets of animals were protected as the dosage was excessive. In the second experiment, when one and one-and-one-half minute exposures were employed, all the white but none of the black rats were protected. The black rats showed rickets by x-ray and by pathological examination, and their blood contained a less percentage of inorganic phosphate. This experiment shows that pigment retards the rays which are effective in rickets, and indicates one factor in the exceptional susceptibility of negro infants to this disorder.

ULTRA-VIOLET RADIATION—WHITE AND BLACK RATS.

| | Wgt. | U.-V. Ray | Diet | X-Ray | Path. | Inorg. P. Mg. per cent. |
|---|---|---|---|---|---|---|
| White.... | 70–70 | 1 min. | 84[3] | Neg. | Neg. | |
| " .... | 58–60 | " " | " | " | " | |
| " .... | 60–70 | " " | " | " | " | |
| " .... | 60–70 | 1½ " | " | " | " | |
| " .... | 60–80 | " " | " | " | " | 5.45 |
| " .... | 64–70 | " " | " | " | " | 4.44 |
| Black.... | 50–60 | 1 " | " | R. | R. | |
| " .... | 50–60 | " " | " | " | " | |
| " .... | 60–58 | " " | " | " | " | |
| " .... | 50–50 | 1½ " | " | " | " | 2.92 |
| " .... | 48–54 | " " | " | " | " | 3.00 |
| " .... | 60–60 | " " | " | " | " | |

Prolonged exposure to direct sunlight failed to prevent or to delay the onset of scurvy in giunea-pigs.

### 105 (1852)

## A note on the preparation of anti-colon streptococcus serum.

By **JOHN F. ANDERSON.**

[*From the Squibbs' Laboratory, New Brunswick, N. J.*]

About two years ago, Dr. H. A. Cotton requested that I should undertake for him the preparation of a combined Anti-Colon Streptococcus Serum prepared from horses treated with cultures of *B. Coli* and *Streptococci* isolated from cases under treatment at the New Jersey State Hospital, Trenton, N. J.

A certain number of cases under treatment at that institution have been found to yield pure cultures of streptococci of diverse types and also strains of *B. Coli.* These organisms are obtained from material removed at operation such as tonsils, extracted teeth, lymph glands, especially those found in the mesentery of the colon, and other material from the abdominal cavity.

The treatment of the horse was with eleven strains of *B. Coli* and eight strains of streptococci, most of which belonged to the hemolytic group.

The immunization was begun with small doses of the killed organisms, but after several weeks the use of live cultures was adopted. It was found that the injections of the mixed antigens of the two bacteria were followed in some instances by severe reactions in the horse and that when the antigens were given on different days that the colon antigens were not tolerated as well as the streptococcus antigens. The injection of the colon bacilli induced such violent reactions in some instances that recourse was finally had to the method of preparing the antigen suggested by the author for the preparation of anti-meningococcic and anti-pneumococcic sera.

The plan of treatment as finally decided upon was the injection of washed broth cultures of streptococci and washed agar cultures of colon bacilli. The two antigens were suspended in salt solution and given intravenously for four injections on successive days followed by a rest period of three days when the injections with gradually increasing amounts were repeated.

After the horse had been under treatment for about 8 weeks, a trial bleeding was made and the titre of the serum determined by the use of the agglutination test as used by the Hygienic Laboratory for the testing of Anti-Meningococcic Serum. Based on the results of the tests of the trial bleedings, an adjustment was made in the proportion of the individual components of the mixed antigen in order that the serum should be as well balanced as possible.

It was found, however, that in spite of all efforts to promote the formation of antibodies to each strain of bacteria used in the injection of the horse that agglutinins, at least, were never produced in measurable amounts to a few strains. This of course is not unique as many have found that some strains of bacteria do not induce antibody formation.

A few weeks after the trial bleeding the horse was bled for production by taking 6 liters of blood six days after the last injection and 6 liters 48 hours later. The serum from the two bleedings was combined, four tenths of one per cent. tricresol added, filtered through Berkefeld filters, tested for sterility and for certain antibodies.

It was found that the serum agglutinized all of the strains of *B. Coli* except two in dilutions from 1–100 to 1–400 and that all of the strains of streptococci were agglutinated in dilutions from 1 to 200 to 1 to 400.

In addition complement fixation tests with the serum gave fixation but not to a high degree with both the hemolytic and non-hemolytic groups of streptococci.

Protection tests in animals have not been made but it is believed that sufficient data has been accumulated in the literature to justify the advance of the opinion that a properly prepared anti-streptococcic serum will afford a considerable measure of protection against streptococci if given 12 to 18 hours before the bacteria are injected. There is not the same data for an anti-colon serum as but few attempts have been made to make and use such a serum.

While most are agreed as to the possibility of giving protection to animals by the previous administration of an anti-streptococcic serum, there is considerable difference of opinion as to the curative value of such a serum, although the weight of opinion is in its favor·

Some of the failures of anti-streptococcus serum may have been due to the fact that the particular strain of streptococcus causing the infection was not among those groups used for the immunization of the horse. The importance of this was not realized until comparatively recently and it is possible that further work may show that a potent anti-streptococcic serum can be prepared for each strain.

The pathogenicity of the colon bacillus for laboratory animals shows much variation as the intravenous administration of some strains in small amounts sometimes quickly results in death, while large amounts of other strains are well borne.

The symptoms and fatal results are probably due to the action of toxins or poisons contained in the body of the bacteria.

The colon bacillus has been claimed to be the cause of a variety of conditions in various parts of the body, particularly in the region of the abdominal cavity, but some observers have thought it questionable if the colon bacillus was the primary cause of the lesions due chiefly to the observation that it is not always found in pure culture.

The growth of the colon bacillus is not attended with the production of a soluble toxin or poison of a high degree and therefore the injection of filtrates into animals of broth cultures is not followed by marked antibody formation, but if animals such as the rabbit or horse are given gradually increasing doses of killed or live cultures there quickly appear in their blood antibodies such as bacteriolysins, agglutinins, precipitins, and complement-fixing substances.

The fact that antibodies are produced in high degree in immunized animals gives support to the opinion of Dr. Cotton that a combined Anti-Colon Streptococcus Serum is of value in preventing post-operation infections due to colon bacilli or streptococci and perhaps may be of value in the treatment of infections by those organisms already developed.

### 106 (1853)

## The use of a colon-streptococcus anti-serum as a pre-operation measure.

By **JOHN WILLIAM DRAPER.**

[*From the State Hospital, Trenton, N. J.*]

The work here presented is a part of the elaborate research into the causation and treatment of the so-called functional psychoses, made at the State Hospital at Trenton, New Jersey, under the direction of Dr. Henry A. Cotton.

More than thirty years ago, while performing autopsies upon the bodies of patients dying in the hospital for the insane at Chicago, Dr. Albert J. Ochsner[1] noted that there was present in an unusually large number very marked pathological changes in the colon, and occasionally in the other viscera. He called the attention of the authorities to these findings but was told that even if present, these lesions had nothing whatever to do with the psychosis, which was a personality or psychic disorder, entirely separate and bearing no relation whatsoever to any physical defects which might be present. In spite of his protestations this opinion prevailed. Undoubtedly many other pathologists and surgeons, both here and abroad, have noted the striking frequency with which extensive pathological changes in the abdominal portion of the alimentary canal and elsewhere are to be noted among patients suffering with the so-called "functional" psychoses.

Impressed with the very definite clinical improvements which he had obtained by removing dental and tonsillar foci of infection among these patients, and believing that there must be additional sources for the very evident toxemia among those who made little or no improvement after the removal of these oral infections, Dr. Cotton invited me to conduct a surgical research which should furnish evidence as to the presence or absence of such abdominal infection. This work is now entering upon its fourth year. The pathology is present and the favorable clinical results following its removal are already rather widely known. Suffice it here to say, that as a result of the application of the usual surgical principles of detoxication by elimination of all foci within reach, the hospital discharge rate has risen from thirty-five to seventy-six per cent.[1]

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[1] Ochsner, Albert J., personal communication.

Over ninety per cent. of all patients classified as "functional" psychotics have marked oral infection. Twenty per cent. present marked evidence of gastro-intestinal disease. Until some better form of therapeusis or early prevention can be found, there seems no better method at hand, as stated by Dr. James Ewing, than surgical removal. Continuing in a report of sixteen specimens of colon and ileum from this series, Dr. Ewing says:

"The most marked and constant lesion is pigmentation of the mucosa which has rendered the inner lining brownish or at times dark chocolate in color. This change is most marked in the cecum, diminishing toward sigmoid, but often present throughout the specimen. Sections show the pigment to be lodged in large polyhedral cells lying in the mucosa and at times in the epithelium. Pigmentation of the colon is fully recognized as a sign of chronic intestinal stasis and intoxication. It is sometimes associated with anemia and at times with severe and even fatal dystrophies of nervous and muscular systems."

"Pouching of the intestinal wall amounting almost to hernial protrusions was observed in most of the cases. These pouches were from one to two cm. in depth. The wall of the pouches was generally thinned, sometimes very much thinned, and the mucosa at the bottom was generally eroded, sometimes ulcerated. Through such erosions and ulcerations it is obvious that absorption of fluids and bacteria readily occurred."

"In general, the impression gained from the study of these specimens was that the clinicians were dealing with extensive and somewhat unusual grades of chronic intestinal stasis and catarrhal inflammation with its sequels."

In 1919, thirty-four partial resections of the colon were made with thirteen deaths or 40 per cent. mortality. In 1920, fifty-nine partial resections were done with eighteen deaths or 30 per cent. mortality. In 1921, forty-six partial and thirty-one total resections were done with ten deaths or 12 per cent. mortality. It is a very simple matter to account for the lowering of the mortality rate from 40 to 30 per cent. It embraced the training of the staff and the development of an improved surgical technique. The fall from 30 per cent. to 12 per cent. followed immediately upon the introduction of the serum treatment, all other factors remaining as before. Coincident with the 30 per cent. mortality among the insane, the writer's mortality among private cases was 17.7 per cent., showing that psychotic patients are not good surgical risks. They are all physically sick. Their systolic blood pressure is abnormally low, and, as is well known, they often have an abnormally high small lymphocyte count coupled with a very small number of polymorphonuclear cells, the former sometimes exceeding the latter.

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[1] Cotton, H. A., "The Defective Delinquent and Insane," 1921.

Aside from the extensive pathological lesions in the mucous membrane and the wall of the colon, which were found in the specimens removed at operation, it was noted that the mesenteric lymph nodes were very much enlarged. In the very beginning of the work these lymph nodes were cultured and various strains of streptococci and colon bacilli were isolated. This finding was of the utmost importance as it clearly indicated that these bacteria were passing through the wall of the intestine and were in all probability the cause of the lesion in the intestinal wall.

When these enlarged nodes were found in the mesentery of the colon alone, resection was clearly indicated. In many cases, however, it was found that the adenitis was not limited to the mesentery of the colon, but extended throughout the whole mesentery of the small intestine. In such cases it was evident that removal of the colon would not correct or eliminate the evident lesion throughout the whole of the small intestinal tract.

It was necessary, therefore, to devise some other method whereby these infections could be mitigated or eliminated. Autogenous vaccines made from the streptococci and colon bacilli isolated from the lymph nodes, were tried, but without success, probably because of the extent and severity of the infection existing in the intestinal wall, a condition analogous to that found in typhoid fever. As is well known, typhoid vaccine will immunize a patient against typhoid fever, but once the disease is established the vaccines have no value in the treatment, because of the overwhelming number of typhoid bacilli in the intestinal tract.

When it was found that the autogenous vaccines were not effective another method had to be devised. As no colon serum had been previously made, Dr. Cotton consulted with Dr. John F. Anderson, who agreed to attempt to immunize a horse with strains isolated in the laboratory at the State Hospital at Trenton. It was found that the colon bacilli, even in small doses, were extremely toxic for the horse, but finally a serum was produced which was very potent.

As many of the patients were suffering from combined infection of the streptococci and colon bacilli, it was decided to combine these organisms in one serum, merely for the sake of convenience of administering the serum. As noted below, this serum has proved entirely satisfactory, not only in reducing the surgical mortality, but, combined with the surgical procedure of removing the infected colon, it has apparently hastened the disappearance of the mental symptoms immediately following the operation.

Hence, the colon-streptococcus anti-serum, prepared from strains furnished by Dr. Cotton, has been given as a pre-operative treatment, in a series of from eight to ten doses, extending over about a month. Without any other change being made in the surgical technique or post-operative care, the mortality dropped to 12 per cent. Autopsy notes show that this decrease was primarily due to a great reduction in the number of perforations which formerly had occurred in the operation area. It has been noted, clinically, that the character of the improvement is transient, probably due to reinfection.

This series of one hundred and seventy cases of colectomy is too small to permit of any definite deductions as to the value of this serum, and this report is merely a provisional one, the encouraging nature of which may not be supported by subsequent studies.

### 107 (1854)

## Spontaneous agglutinability of bacteria in relation to the antagonistic action of certain cations.

By RALPH R. MELLON.

[*From the Department of Laboratories, Highland Hospital, Rochester, N. Y.*]

Spontaneous agglutinability of five separate, single-celled (pure line) cultures of diphtheroid bacilli was shown to be a function of growth-cycle developments. The bacillary phase, growing at 37°, was immediately and completely agglutinable by any solution tried. The coccus phase, growing at 20°, formed stable emulsions in NaCl and other salt solutions. By reversing the growth temperature, even on the same media, the agglutinability and morphology were reversed. Certain of the cultures, still completely agglutinable by NaCl, formed stable emulsions in Tyrode's and other equilibrated solutions. The mutual antagonism of the Na, K and Ca ions is believed to explain the phenomenon. The Mg ion was especially beneficial. With various cultures of different age and environment, all possible variations in agglutinability were observed. The amplification of these observations, now in progress, promises to explain some of the paradoxes of the bacterial agglutination, and the observations in themselves constitute the first systematic application of these principles to agglutination.

### 108 (1855)

## Blood pressures and heart rate, in girls, during adolescence. A preliminary study of 1,700 cases.

By STANLEY ROSS BURLAGE (by invitation).

[*From the Department of Physiology, Cornell Medical College, Ithaca, N. Y.*]

The data were obtained from over 800 girls in the public schools of Ithaca, N. Y., whose ages ranged from 9 to 16 years and from about an equal number of young women in Cornell University, from 16 to 26 years old.

Blood pressures were taken by Korotkow's auscultatory method, using in all cases a Princo mercury sphygmomanometer. The reading for the diastolic pressure was made at the beginning of the fourth phase. All girls were examined in the sitting posture.

There is a rapid rise in the systolic pressure from 104 mm. at 9 years to approximately 124 mm. at 14 years. This remains at the same level through the next year. Then there is a rapid fall of over 10 mm. to 18 years. From that age on the pressure remains fairly constant around 110 mm. up to 26 years.

The diastolic pressure rises evenly from 63 mm. at 9 years to about 76 mm. at 14 years. It maintains about this level throughout the remaining years.

The pulse rate drops rapidly from 98 at 9 years to 80 at 18 years and then continues with little change.

Since, at 14 years of age, practically all girls in this climate have begun to menstruate, these curves would seem to indicate, that allowing 3 or 4 years for recovery from metabolic disturbances incident to the onset of puberty, the blood pressures and pulse rate vary little during the following 8 years.

In the height curves, the systolic pressure rises gradually from 104 mm. at 50 inches to 113 mm. at 69 inches. The diastolic pressure rises slightly more rapidly—from 64 mm. to 74 mm. The pulse rate drops rather evenly from 106 to 82 per minute.

The weights are arranged in classes of 10 lbs. each.

The systolic pressure rises rapidly from 104 mm. for the 51–60 lb. class up to 118 mm. for the 91–100 lb. class. Then it runs along without much change until it reaches the 151–160 lb. group. Here the rise is abrupt up to about 130 mm. for the 200 lb. class. The diastolic pressure shows a gradual rise from 58 mm. in the 41–50 lb. class to about 90 mm. at 200 lbs.

The pulse rate shows a decline from 102 at 51–60 lbs. to 78 at 200 lbs.

The conclusion from a consideration of these data is that in determining the normal blood pressures and pulse rate for girls between 9 and 26 years of age, it is necessary to consider, not age alone, but weight and height as well.

### 109 (1856)

## Serological studies of the diphtheria group.

By **CARL O. LATHROP** and **CHARLES A. BENTZ.**

[*From the Bacteriological Laboratories of the Medical Department, University of Buffalo, and Buffalo Bureau of Laboratories, Buffalo, N. Y.*]

A systematic qualitative and quantitative study has been made on the immunity developed in a group of young adults immunized against diphtheria with toxin-antitoxin.

As a preliminary, a successful attempt was made to corroborate Havens' contention that there are two serological groups of diphtheria bacilli with specific agglutinogenic properties and no evidence of cross agglutination. For our minor group antigen we used two cultures recovered from cases of diphtheria developed in persons immunized with toxin-antitoxin and yielding negative Schick tests.

This specificity has been further substantiated by antibody absorption, which confirms the other findings completely.

Incidentally, we used rabbit blood agar plates exclusively for isolation and study of the organisms, and noted that hemolysis, a sometime mooted point, is not characteristically allied with virulence, nor does it only occur in freshly isolated cultures, but may crop up as late as the 56th generation.

We have also found, as we believe Park stated, that certain true diphtheria bacilli possess a factor of virulence not neutralized by antitoxin, concerning whose identity we are making a further study. Likewise we have confirmed Park and Havens in finding that there is some group antitoxin present in the antitoxin commonly in use for the toxin of the minor group.

Using a modified Römer method, we titrated the antitoxin content of a group of young adults immunized against diphtheria with toxin-antitoxin. We found 20 out of 26 had developed antitoxin in quantities varying from 1/30 unit up to 1/5, while three developed only 1/50, and three failed to develop any immunity.

We then ran Schick tests with regular and minor group toxins.

Without exception, all gave a strongly positive reaction to the minor group toxin, though 20 were protected completely against the regular toxin, and three more partially so.

To further verify this stage of the work, we tried titrating any possible group antitoxin against the minor group by using a ripened minor group toxin, whose standardization, for obvious reasons, could not be attempted with standard antitoxin. Although we worked at the limit of sensitivity, approximately 1/500 M.L.D., we were unable to demonstrate the presence of any protective power in the blood of any of this group against the minor toxin. Seemingly, then, immunization with monovalent diphtheria toxin-antitoxin does not protect against infections of the minor group, as, of course, is manifest in the two cases of that type originally quoted and with whose cultures we started our work.

Two of the most important laboratories in the country have recently told us that they were likewise studying the toxins of certain diphtheria organisms which are not neutralized by the antitoxin in current use, so we hope after their publication that steps will be taken to include the minor group in the preparation of antitoxin, toxin-antitoxin, and Schick test toxin.

### 110 (1857)

## An intramuscular method of digitalis assay.

By **M. S. DOOLEY** and **C. D. HIGLEY** (by invitation).

[*From the Pharmacological Laboratory, Syracuse University, Syracuse, N. Y.*]

Many observers have criticized the one-hour method of standardization on account of the failure of complete absorption.

During the course of work on the elimination of digitalis substances, one of us demonstrated the feasibility of making intravenous injections in the frog by the insertion of a fine hypodermic needle into the abdominal vein. It was suggested by Dr. Hatcher that an intravenous method of assay might be evolved upon the frog. Efforts to do so have not been successful but in testing the possibility the idea occurred to us to experiment with an intramuscular method.

Our method is to inject one half the total dose into the thickest part of each thigh, the needle being directed diagonally. The finest needle must be used and hemorrhage avoided. Otherwise the procedures are the same as for the Pharmacopeial method.

Judging by our results, the intramuscular method very largely and, we believe, satisfactorily, solves the difficulty of poor absorption.

TABLE I.

Crystalline Strophanthin (Ouabain).

| Frogs. | S. S. S. Dose in Mg./Gm. | | Per Cent. Difference. |
|---|---|---|---|
| | Lymph Sac. | Intramuscular. | |
| Lot 1 | .00045 | .00037 | 17.8 |
| " 2 | .00051 | .00044 | 13.8 |
| " 3 | .00032 | .00027 | 15.7 |
| " 4 | .00039 | .00031 | 20.6 |
| " 5 | .00059 | .00050 | 15.3 |
| " 6 | .00057 | .00051 | 10.6 |

Digitalis-Tinctures.

| Frogs. | Lymph Sac. | Intramuscular. | Per Cent. Difference. |
|---|---|---|---|
| Lot 1 | .70 | .57 | 18.6 |
| " 2 | .59 | .49 | 17.0 |

Digitalis-Fluidextracts.

| Frogs. | Lymph Sac. | Intramuscular. | Per Cent. Difference. |
|---|---|---|---|
| Lot 1 | .90 Neg. | .70 | 22.3 |
| " 2 | .60 | .50 | 16.7 |

Digitalin-Merck.
Formerly Known as "German."

| Frogs. | Lymph Sac. | Intramuscular. | Per Cent. Difference. |
|---|---|---|---|
| Lot 1 | .025 | .022 | 12.0 |

Table I summarizes the results. In all cases the intramuscular S.S.S. (systolic standstill) dose has been compared with the lymph sac S.S.S. dose. In no instance has the intramuscular dose been found as large as that by the lymph-sac method.

Tinctures, fluidextracts, digitalin and ouabain have been studied. The last column of the table shows the percentage differences between the lymph sac and the intramuscular S.S.S. doses in corresponding lots of frogs. The effective intramuscular dose of digitalin is 10 per cent. less than by the lymph sac while, with tinctures and fluidextracts, the difference is an average of 18.6 per cent. The intramuscular method gives more constant end points and, therefore, requires less time and material for an assay. Lot I, fluidextracts, illustrates this point. With this preparation we were unable to determine the effective dose on account of poor absorption even with injections of doses 20.6 per cent. above the intramuscular dose. Even ouabain, generally considered as satisfactorily absorbed from the lymph sac, has required an average dosage 15.6 per cent. less by the intramuscular method. Earlier experiments have been repeated at a different season on different lots of animals with similar results.

It is believed that division of the dose, better blood supply in muscle than in skin and massage from movements of the animal, account for the more constant results and for the smaller intramuscular dosage required.

## ABSTRACTS OF THE COMMUNICATIONS, MINNESOTA BRANCH.

*Minneapolis, Minnesota, February 8, 1922.*

### Third Meeting

### 111 (1858)

### Evidences of a structure in gelatin gels.

By **ROSS AIKEN GORTNER** and **W. F. HOFFMAN.**

[*From the Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

Bancroft[1] recently reported some conclusions drawn from unpublished data of a Mr. Cartledge who dried gelatin gels of different concentrations down to a 96 per cent. gelatin content and then allowed these dried sheets to again imbibe water. It was found that "each swelled rapidly to the original concentration and then took up water slowly."

We have conducted experiments similar to those of Cartledge and have secured comparable results. Thus a 10 per cent. gelatin gel dried down to less than 3 per cent. moisture content had imbibed at the end of 72 hours 6.45 grams of water per gram dry gelatin as contrasted with 4.30 grams water for a 40 per cent. gel similarly treated. Comparable differences were observed when the dried sheets were ground and uniform sized particles sieved out and tested for hydration rate and maximum hydration capacity.

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[1] Bancroft, W. D., "Applied Colloid Chemistry," 1921, p. 251.

Our experiments indicate that gelatin gels have a structure and that this structure is fixed at the time of gelation and is not appreciably altered by drying the gel at room temperature. A crystal structure in which the gelation temperature is actually the melting point of the crystals would explain the peculiarities observed.

### 112 (1859)

## The control of respiration.

By **C. C. GAULT** and **F. H. SCOTT.**

[*From the Department of Physiology, University of Minnesota, Minneapolis, Minn.*]

The movements of respiration are carried out by voluntary muscles and ought to obey the laws of voluntary movement. One of the chief points in muscular action is the dependence of motor response on sensory impulses. Without the guidance of sensory impulses movements are ataxic. Just how ataxic or abnormal movements become depends on the extent of loss of sensory impulses and on the ability of the mechanism to guide itself by sensory impulses from other sources. These statements hold true in regard to the movements of respiration. It has long been known that a modified respiration results from cutting off the sensory impulses from the lungs by section of both vagi. Many investigators have, however, kept animals with divided vagi so that one cannot maintain that the vagi are essential to respiration. However, it was pointed out by one of us[1] a number of years ago that animals with vagi divided are not nearly as efficient in times of respiratory stress as is a normal animal. It was shown by

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[1] Scott, F. H., *Jour. of Physiology*, 1908, xxxvii, 301.

Alcock, Einthoven and others that expansion of the lungs sets up electrical variations in the vagus, thus showing these sensory impulses actually exist. A number of years ago we[1] called attention to the alteration in respiration after section of the cord or division of the posterior thoracic roots. That impulses are set up in the joints of the thoracic cage every time the thorax expands may be shown by connecting the peripheral end of a cut intercostal nerve to a string galvanometer. There is an electrical variation each time the thorax is expanded. The respiratory center is thus informed of the position of the thorax as well as the position of the lungs. We will discuss in detail at a later time the effects of these impulses on the respiratory center.

One of the means of testing ataxic muscle is to have it do certain movements more strenuous than normal. The respiratory mechanism may be tested in this manner with either increased $CO_2$ or decreased $O_2$ in the air. We finally adopted the method of rebreathing. By this method we found animals with divided vagi or with divided cord are much less efficient than normal animals. In a number of cases we found in animals with divided cord that rebreathing from the spirometer caused a marked slowing of respiration. The respirations always increased in depth but the animals were unable to make a deep and at the same time a rapid respiration. In no case after division of the cord did we find an increase in rate nearly proportional to that in the intact animal. As examples two experiments may be quoted:

| | Rate. | Vol. | Total c.c. per Min. |
|---|---|---|---|
| Cat, normal | 40 | 61.6 | 2464 |
| After 1 min. rebreathing | 50 | 75.6 | 3780 = + 53% |
| Cord cut 7th cervical 17 min. after operation | 37 | 40.7 | 2030 |
| After 1 min. rebreathing | 30 | 47.6 | 1628 = − 19% |
| Cat, normal | 39 | 50.4 | 1965 |
| After 1 min. rebreathing | 48 | 75.6 | 3628 = + 84% |
| 30 minutes after division of vagi | 15 | 75.6 | 1134 |
| After 1 minute rebreathing | 15 | 106.4 | 1489 = + 39% |
| 15 minutes after division of cord at 7th cervical | 15 | 56 | 840 |
| After 1 minute rebreathing | 15 | 70 | 1050 = + 25% |

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[1] Gault, C. C., and Scott, F. H., *Am. J. of Physiology*, 1918, xlv, 555.

### 113 (1860)

## Potassium iodide does not influence the course of an experimental actinomycosis.

By **A. T. HENRICI** and **G. S. REYNOLDS.**

[*From the Department of Bacteriology and Immunology, the University of Minnesota, Minneapolis, Minn.*]

It is generally believed that iodides are almost specific in their favorable influence upon the course of various mycoses, notably sporotrichosis, aspergillosis, "blastomycosis," and actinomycosis. There can be no question about the value of iodides in sporotrichosis, but concerning the other mycoses reports are not so uniform. It is generally believed, however, that the iodides may cure actinomycosis, particularly in cattle.[1]

Some of the fungi are not virulent for lower animals, and most of the others rapidly lose their virulence when cultivated, so that little experimental work has been done. Renon[2] found that *Aspergillus fumigatus* grew in culture media containing as much as 10 per cent. of potassium iodide; but that inoculated rabbits treated by subcutaneous injections of the salt did not die until 26 and 32 days after infection, whereas the control died in 4 days. Davis[3] found that in experimental sporotrichosis the injection of iodides previous to or simultaneous with inoculation had no inhibiting effect on the course of the disease; but when administered after the infection is under way, the lesions heal. He also found that *Sporotrichum* would grow in media containing considerable quantities of iodide.

Henrici and Gardner[4] have isolated from a case of pulmonary infection a variety of *Actinomyces* very similar to but not quite identical with *A. asteroides* Eppinger, which they named *A. gypsoides.* This fungus is very virulent for guinea pigs, and has maintained its virulence quite unaltered for several years. It is an acid-fast variety quite different from *A. bovis*, and there are no clinical reports of the use of iodides in this type of actinomycosis. Nevertheless, because of its constant virulence it is admirably suited for chemotherapeutic experiments, and it was thought desirable to see what influence iodides would have on the course of the infection in guinea pigs.

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[1] Salmon, D. E., Eighth and Ninth Annual Reports of the Bureau of Animal Industry, Washington, 1893.

[2] Renon, L., "Etude sur l'Aspergillose chez les Animaux et chez l'Homme," 1897.

[3] Davis, D. J., *Jour. of Inf. Dis.*, 1919, xxviii, 124.

[4] Henrici, A. T., and Gardner, E. L., *Jour. of Inf. Dis.*, 1921, xxviii, 232.

The potassium iodide was administered by mouth in aqueous solution. The results are shown in the table below. The first guinea pig was treated with iodides alone; animals 2 and 4 received iodide both previous and subsequent to inoculation; animals 3 and 5 are untreated controls to 2 and 4 respectively.

| Date. | Guinea Pig 1. | | Guinea Pig 2. | | Guinea Pig 3. | | Guinea Pig 4. | | Guinea Pig 5. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Wt. gm. | KI, gm. | Wt. gm. | KI, gm. | Wt. gm. | KI, gm. | Wt. gm. | KI, gm. | Wt. gm. | KI, gm. |
| Jan. 4..... | 743 | 0.12 | 838 | 0.25 | | | 654 | 0.01 | | |
| Jan. 6..... | 695 | 0.25 | 790 | 0.25 | | | 622 | 0.25 | | |
| Jan. 9..... | 675 | 0.25 | 743 | 0.25 | 1012 | 0 | 620 | 0.25 | | |
| | | | (inoculated) | | (inoculated) | | | | | |
| Jan. 11.... | 650 | 0.25 | 625 | 0.25 | 965 | 0 | 595 | 0.25 | | |
| Jan. 12.... | | | died | | died | | | | | |
| Jan. 13.... | 608 | 0.25 | | | | | 565 | 0.25 | | |
| Jan. 16.... | 580 | 0.25 | | | | | 543 | 0.25 | | |
| Jan. 18.... | 572 | 0.25 | | | | | 539 | 0.25 | — | 0 |
| | | | | | | | (inoculated) | | (inoculated) | |
| Jan. 20.... | 580 | 0.25 | | | | | 555 | 0.25 | 560 | 0 |
| Jan. 21.... | 562 | 0.25 | | | | | 500 | 0.25 | — | 0 |
| Jan. 23.... | — | — | | | | | 438 | 0.25 | 485 | 0 |
| Jan. 24.... | 600 | 0.25 | | | | | died | | died | |

It will be seen from the above that the treated animals succumbed simultaneously with the controls; and that the iodide itself, while given in relatively large doses, was not sufficient to hasten death.

*In vitro* it was found that both *A. gypsoides* and *A. asteroides*, while retarded, still grew in broth containing 10 per cent. of potassium iodide.

It is clear, then, that potassium iodide has no specific action on this type of *Actinomyces*, and if it has any clinical value, it is due, as in sporotrichosis, not to an action on the parasite itself, but because of its action in stimulating the formation of granulation tissue. With *A. gypsoides* the course of the disease is too rapid to demonstrate this latter point in the guinea pig.

114 (1861)

## Evidence of a structure in gelatin gels.[1]

By **ROSS AIKEN GORTNER** and **W. F. HOFFMAN.**

[*From the Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

The question as to whether or not a colloid gel has a definite structure is extremely important to the biologist because of its bearing on the structure of protoplasms.

Bancroft[2] recently reported some unpublished results of a Mr. Cartledge in which gelatin gels of different concentration were dried down to 96 per cent. gelatin and then these dried sheets were allowed to again imbibe water. It was found that "each swelled rapidly to the original concentration and then took up water slowly," or in other words, a film of dried gelatin made from 8 per cent. gelatin gel was still potentially an 8 per cent. gel and very different from a dried film from a 16 per cent. gel. Arisz[3] had previously reported data showing a marked difference in imbibition capacity of gelatin gels of different concentrations and also of discs of uniform concentration but of different thickness.

Inasmuch as the experiments cited by Bancroft have such a profound bearing on all colloid-chemical studies in which gelatin has been used, it appeared worth while to attempt to duplicate Cartledge's results. Accordingly a series of gels was prepared as follows:

Weighed quantities (10, 15, 20, 25, 35 and 40 grams) of "Bacto" gelatin[4] were added to 100 c.c. of distilled water in clean pyrex flasks. After soaking for 15 to 30 minutse the flasks were placed in a hot water bath and allowed to remain until all of the gelatin had dissolved to form a homogeneous solution. A measured quantity (25 c.c.) of this solution was then poured into petri dishes 89 mm. in diam., thus ensuring the same thickness of the gelatin gel in each instance. After standing for 12 hours duplicate rectangles 5 x 2.5 cm. in surface area were cut and placed on watch glasses to dry in a current of warm air (30°–40° C.) to a moisture content which did not exceed 3.5 per cent. The rate of moisture loss was followed by frequent weighings of the gelatin plates during the drying process, but no marked differences in rate of moisture loss were observed. The dried sections were then placed

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[1] Published with the approval of the Director as Paper No. 311, Journal Series, Minnesota Agricultural Experiment Station.

[2] Bancroft, W. D., "Applied Colloid Chemistry," 1921, p. 251.

[3] Arisz, L., *Koll. Beih.*, 1915, vii, 51–6.

[4] Air-dry gelatin as received from manufacturer.

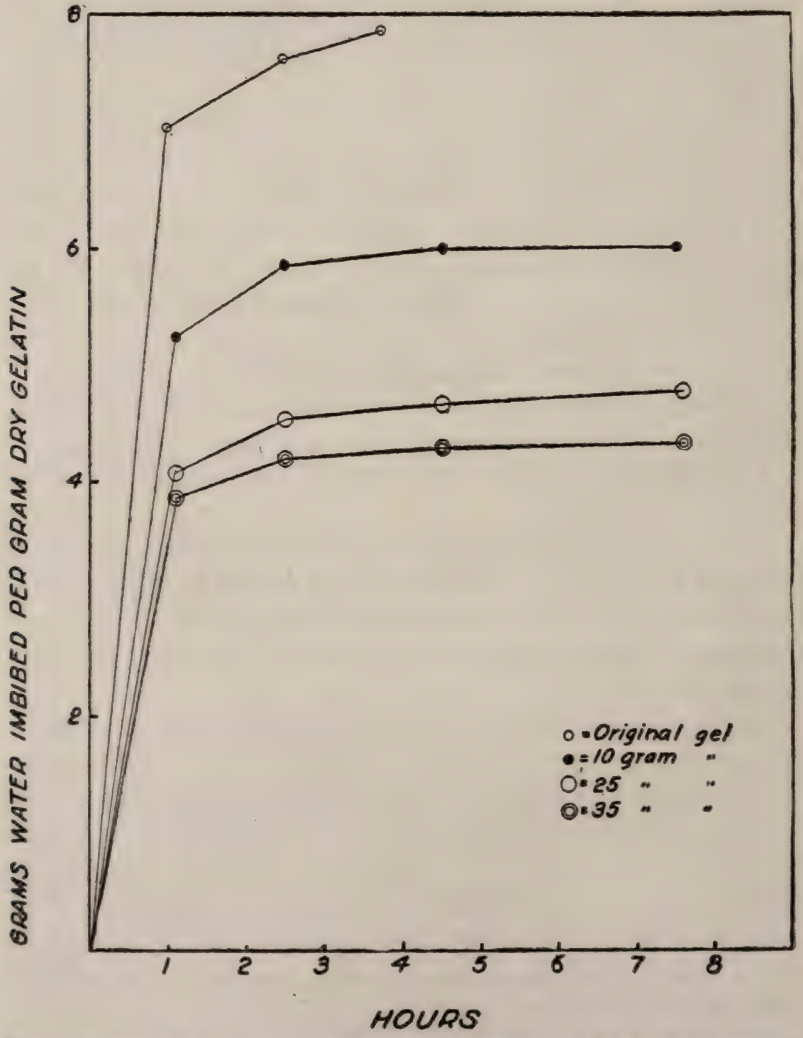

FIG. 1. Showing imbibition curves (in water) for sections of dried gelatin prepared from gels of different initial concentrations.

in distilled water and allowed to reimbibe moisture. The rate of swelling was followed by weighing the discs at frequent intervals after removing surface water by blotting with neutral filter paper. The data calculated in *grams water imbibed per gram dry gelatin* are shown in Fig. 1.

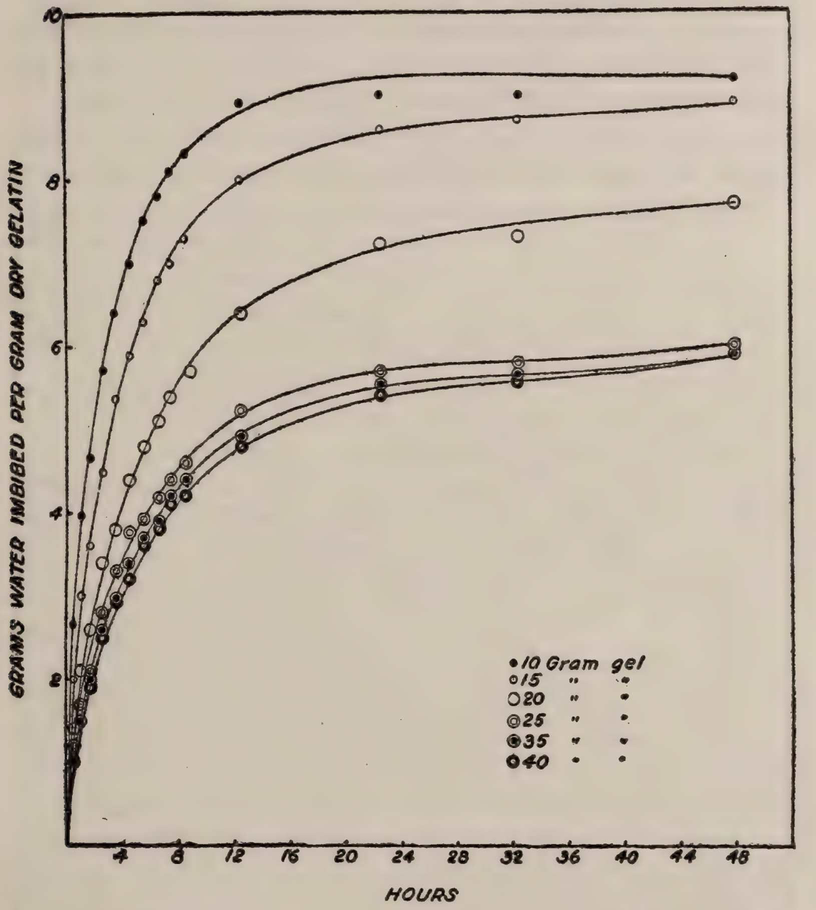

FIG. 2. Showing imbibition curves (in water) for dried sections of a 10 per cent. gel and a 40 per cent. gel. Dried sections of equal surface area and of equal thickness.

While these curves appear to indicate marked differences in the dried plates, depending on the original concentration of the gel, such a conclusion is open to the criticism that the dried plates, while of equal surface area, were necessarily of unequal thickness.

We therefore prepared another series of 10 per cent. and 40 per cent. gelatin gels (10 grams air-dry gelatin in 90 grams water and 40 grams gelatin in 60 grams water) and poured the 10 per cent. gel 4 times as deep in the petri dish as was the 40 per cent. gel. These on drying should give gelatin plates of equal area and thickness. After drying these plates they were allowed to imbibe water as in the above experiment. Figure 2 shows the imbibition curves obtained. Here again there is a marked effect due to the initial concentration of the gel. Similar discs were placed in *N*/25 lactic acid ($P_H$ 2.49). It is well known that acid solutions greatly influence rate of imbibition and amount of swelling of

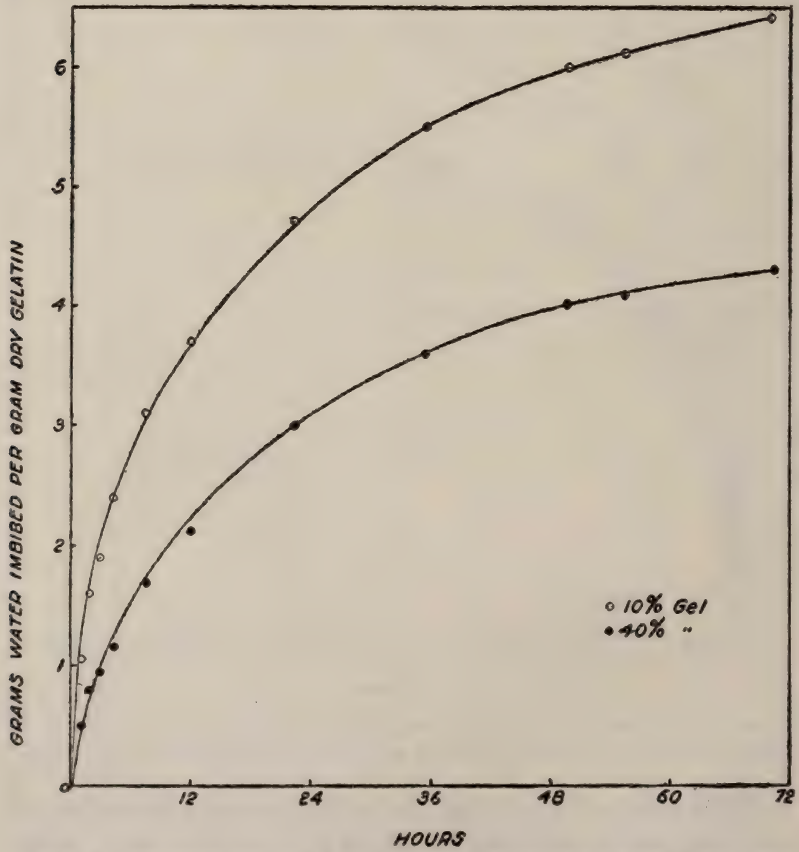

FIG. 3. Showing imbibition curves (in *N*/25 lactic acid) for dried sections of a 10 per cent. gel and a 40 per cent. gel. Dried sections of equal surface area and equal thickness.

proteins on the acid side of the isoelectric point. Fig. 3 shows the very marked differences which were obtained.

In order to be perfectly sure that the shape or thickness of the gelatin plate was not responsible for the differences in swelling, portions of dried plates from 10-gram, 25-gram and 35-gram gels, as well as portions of the "original" Bacto gelatin, were ground and sieved, those particles passing through a 2-mm. sieve and remaining on a 1-mm. sieve being retained for experimental work. A weighed quantity of these "granules" were placed in a Gooch crucible and allowed to imbibe water. At frequent intervals the crucibles were removed from the water, centrifuged at low speed for 2 minutes in order to remove excess moisture and weighed. Fig. 4 shows the form of the imbibition curves.

In order to ascertain whether the lower imbibing capacity of the gels containing the most gelatin might be due to the dehydrating effect of electrolytes in the gelatin "ash," which of course would be present in increasing amount as the concentration of the gelatin increased, a series of experiments was conducted in which the swelling plates were allowed to imbibe in (1) the same portion (100 c.c. of distilled water), and (2) in frequent changes of distilled water. If electrolytes were present they should have been dialyzed out in series (2). No differences in excess of experimental error were observed; in fact, a slightly greater imbibition was noted in series (1).

The effect of hydrogen-ion concentration upon the physical state of gelatin has been pointed out by many investigators, but it is not clear that we are dealing with any appreciable changes in hydrogen-ion concentration. It is impossible to secure by direct measurement the hydrogen-ion concentration of the dried gelatin plates which are immersed in the distilled water, and we have no means of being sure that it is identical with that of the gel from which the plates were prepared. Electrometric measurements on a 5 per cent. gel gave a $P_H$ of 5.19 and the same value was obtained for the 10 per cent. gel. The higher concentrations of gelatin were so viscous that electrometric determinations were not attempted. Inasmuch as there was no change between the 5 per cent. and 10 per cent. gel we believe that it is safe to assume an initial $P_H$ of approximately 5.2 for all gels before drying. This is slightly on the alkaline side of the isoelectric point of gelatin.[1]

The hydrogen-ion concentration of the water in which the plates were immersed was probably between $P_H$ 5.0 and 6.0. The water as it came from the still was a fair grade of conductivity water and was free from carbon dioxide and ammonia. Naturally it afterward absorbed some carbon dioxide from the air. Kendall[2] has recently presented data showing the rapidity with which this takes place. According to his work distilled water is about $P_H$ 5.7 when in equilibrium within the carbon dioxide of laboratory air. We have tried a number of times to determine the $P_H$ of distilled water, as used in these experiments, but have failed to secure sharp readings on the potentiometer because of the slight conductance of the water, our values ranging between $P_H$ 5.0 and 6.0.[3] Colorimetric measurements of hydrogen-ion concentration could not give accurate readings due to the fact that the indicators are all acids or bases which have greater ionizing power than has the water which is being measured.

If we assume, therefore, that the hydrogen-ion concentration of the dried gelatin plate is identical with that of the original gel from which it was prepared, *i.e.*, $P_H$ 5.2, and that the distilled water was in equilibrium with the carbon dioxide of laboratory air, *i.e.*, $P_H$ 5.7, we should still have no appreciable effect of hydrogen-ion concentration on our experimental results for *the same water and gelatin were used in all experiments*. We doubt whether the above assumptions as to $P_H$ values are justified, but we do feel that the differences in the swelling of dried gelatin plates which we have described are not due primarily to differences in hydrogen-ion concentration.

*Conclusions.*—The above data appear to indicate that gelatin gels have a structure and that this structure is more or less fixed at the time that gelation takes place. It would appear that the gelatin aggregates or micelles are more and more interlaced at increasing concentrations of gelatin. This structure is apparently not appreciably altered by drying at a temperature below the

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[1] Loeb, J., *J. Gen. Physiol.*, 1918, i, 39.

[2] Kendall, J., *J. Amer. Chem. Soc.*, 1916, xxxviii, 2460.

[3] As a matter of fact the $P_H$ of the distilled water is probably not an important factor since the water is so feebly buffered that a mere trace of acid or alkali will change the hydrogen-ion concentration through a wide range.

"melting" point of the gel. The fact that drying does not markedly influence the gel structure appears to argue against the formation of micelles by the adventitious coming in contact of dispersed particles of gelatin, for certainly many gelatin particles must touch each other in the dried sheet, but apparently they do not cohere to each other with any appreciable force, certainly not with a force at all comparable with the force of coherence between particles or micelles originating at the time of gelation. This

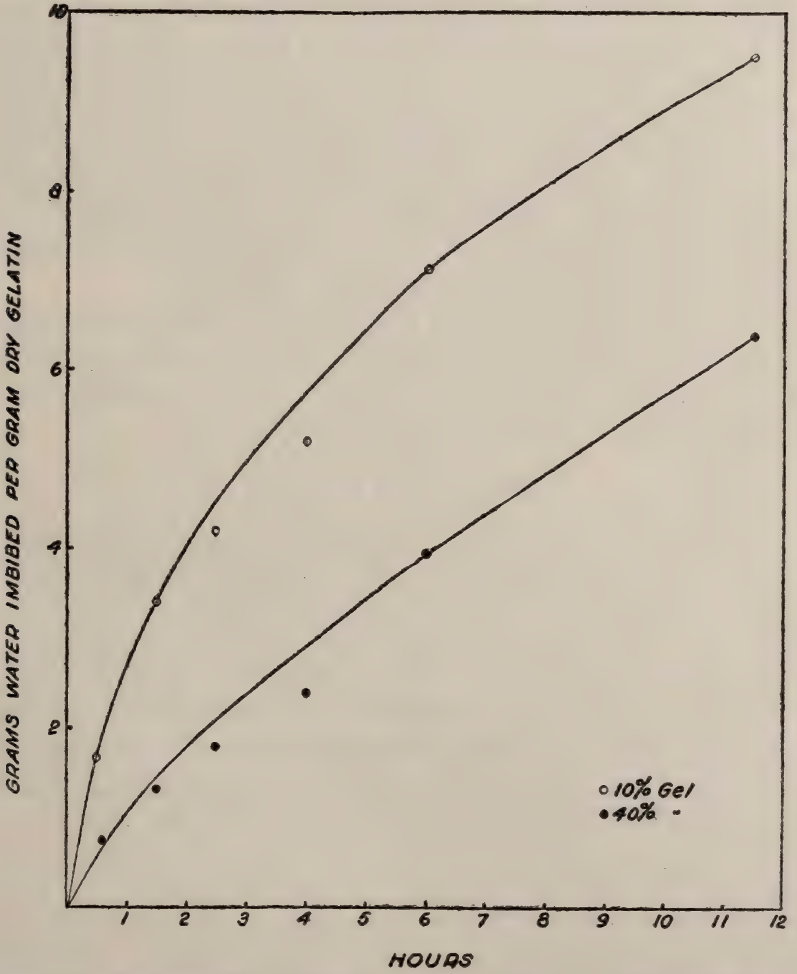

FIG. 4. Showing imbibition curves (in water) for granules of equal average size of dried gelatin prepared from gels of different initial concentration.

might be explained by a crystal structure where the crystals melt or soften at the gelation temperature. The micelles would then be formed by the solidification of crystals and later when micelle touched micelle the hardened surface of the crystal would prevent cohesion. We have recently shown[1] that a crystal gel may be very dilute and yet possess considerable rigidity so that a crystal structure is not incompatible with the properties of gelatin gels.

These experiments likewise show the marked influence that hysteresis may have on experiments where gelatin is involved. This is particularly noticeable in Fig. 4 where uniform-sized particles prepared from gels of different concentration were used. Inasmuch as a dried sheet of gelatin apparently reflects the structure of the original gel from which it was prepared, the production of different samples of dry gelatin possessing uniform physico-chemical properties would appear to be extremely difficult. It is possible that these experiments may account for the differences between the experimental data of various workers.

It would be interesting to know whether alcohol precipitation from sols of differing concentration produces gelatin particles of uniform physico-chemical properties. It is our intention to investigate this problem in the near future.

## ADDENDUM, MARCH 1, 1922.

Since the above MS. was prepared, Sheppard and Elliott[2] have published on the same question. They do not find it necessary to assume a gel structure and believe that the different swelling rates are due to "casehardening" or surface drying effects. We believe that our experiment where uniform sized gelatin particles were employed excludes such an explanation and consequently prefer to adhere to the structure theory outlined above.

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[1] Gortner, R. A., and Hoffman, W. F., *J. Amer. Chem. Soc.*, 1921, xliii, 2199.

[2] Sheppard, S. E., and Elliott, F. A., *J. Amer. Chem. Soc.*, 1921, xliv, 373.

# SCIENTIFIC PROCEEDINGS.

## ABSTRACTS OF COMMUNICATIONS.

### One hundred twenty-second meeting.

*Presbyterian Hospital, March 15, 1922.*
*President Wallace in the chair.*

115 (1862)

### The feeding of non-ketogenic odd-carbon fats to diabetic patients.

By **MAX KAHN.**

[*From the Department of Laboratories, Beth Israel Hospital, New York City.*]

In certain states of the disturbances of the metabolism of fats and carbohydrates a condition of acidosis is established characterized by the fact that the blood is rich in ketonic acids of a certain type. To this condition the special name of "ketosis" has been applied. It is induced by starvation, by the toxic effect of lipin solvent anesthetics, and especially by that disturbance of carbohydrate metabolism known as diabetes.

Under normal conditions, that is, in the presence of proper carbohydrate oxidation, there is a rapid breakdown of the fatty acid fraction of the fats to the four carbon acid, *i.e.*, butyric acid, which is then rapidly catabolized to carbon dioxide and water.

This process is, however, markedly disturbed in states of deficient carbohydrate oxidation. In the latter circumstance the fats are primarily broken down to butyric acid, as in the normal condition, but in the absence of the heat of carbohydrate consumption, the further decomposition of the butyric acid proceeds very gradually. The butyric acid under these conditions is oxidized first to beta-oxybutyric acid, and then to acetoacetic acid, which is decarboxylated to acetone.

It is prohibitive to feed diabetic patients, who have a very low carbohydrate tolerance, even a moderate amount of natural fat, because of the danger of inducing a severe ketosis which may prove fatal. It was thought advisable, therefore, to prepare a synthetic fat to contain fatty acids of odd-carbon number, which, if they are absorbed and if the theory of intermediate fat metabolism described above, holds, should catabolize in the body without the production of the acetoacetic acid, etc.

In collaboration with Dr. H. O. Nolan such synthetic fat was made and fed to typical diabetic and ketotic patients. It was found that the fat was absorbed, that large quantities of it could be fed to these patients without inducing any acidosis, and that the nutrition of such individuals was improved. We are now studying the intermediate metabolism of this fat, and its feeding effect on all types of diabetic and normal individuals.

### 116 (1863)

## Hydrogen-ion concentration studies of solutions used for intravenous medication and clinical investigation.

By **JOHN R. WILLIAMS** and **MADELEINE SWETT** (by invitation).

[*From the Highland Hospital, Rochester, New York.*]

We advance the hypothesis that there is a relationship between the hydrogen-ion concentration of fluids injected intravenously and some of the reactions which follow their use. We base this belief on clinical observations and on extended chemical analyses of fluids commonly used for therapeusis and clinical investigation. We wish to briefly report here some of our more important studies. It should be understood that the hydrogen-ion concentration of normal acid is $P_H$ 2; normal alkali $P_H$ 14; pure water $P_H$ 7; and human blood $P_H$ 7.4, and that this latter figure is fixed and cannot be varied without causing serious disaster or death. If a fluid with a much higher or lower $P_H$ than that of the blood is introduced into the circulation at a rate or in an amount greater than the blood can neutralize or buffer, reactions, as chills, fever and prostration ensue. This is particularly true in individuals whose blood supply is impaired in either quantity or quality.

Since distilled water is the solvent used in most intravenous medication we have studied it with care under the conditions which it is commonly produced in hospitals and laboratories. Rochester tap water has a $P_H$ of 8.29. Boiling was found to increase this slightly. Various samples of stock distilled water varied from $P_H$ 6.89 to $P_H$ 5.05. Distilled water becomes acid even when the distillation is in progress from the absorption of carbonic acid of the atmosphere when the ordinary type of metal or glass still is used.

Tap water, $P_H$ 8.27; first distillate (first 10 c.c.) $P_H$ 7.30; after 200 c.c. had been distilled $P_H$ 6.90. When stored in container, whether sealed with a cotton plug, cork or glass stopper, distilled water becomes quite acid. Example: freshly distilled water has a $P_H$ 6.8, after 48 hours, $P_H$ 5.23. Proximity to bottles containing fuming HCl or $NH_4OH$ made no appreciable difference.

The stock glucose solutions of the hospital prepared from stock distilled water and so-called chemically pure glucose were found to have a $P_H$ of less than 5. Three well-known brands of glucose were tested. Boiling, autoclaving and storing for 24 hours all cause glucose to rapidly become acid. Fresh unheated, 10 per cent. glucose, $P_H$ 6.20; after boiling 10 minutes, $P_H$ 5.47; after boiling 20 minutes, $P_H$ 5.17; after boiling 30 minutes $P_H$ 5. A fresh unheated solution ranged in $P_H$ from 6.2 to 4.15 after standing 48 hours.

Glucose solutions were buffered with the salts of mono- and di-potassium phosphates so as to give a concentration approximating that of the blood. They have been used repeatedly in the hospital without producing reactions. The method of buffering was devised and materials provided by Dr. E. A. Slagle and S. F. Acree. Buffering must be done after sterilization since the latter precipitates out the buffer substances.

The normal salt solutions of the hospital were found to be very acid ranging from $P_H$ 6.4 to $P_H$ 4.95. This was due in part to the use of stock distilled water. One well-advertised brand of normal salt solution put up in ampoule form had a $P_H$ 4.95. This high concentration in normal salt solution was found to be due in part to the salt but chiefly to the acid distilled water. A salt solution to be normal physiologically, should contain that amount of salt which will make it isotonic with the blood. It should also have the same hydrogen-ion concentration.

One specimen of sodium citrate solution used in a transfusion where a violent reaction followed, had a $P_H$ of 10.25. We have had no reactions in seven transfusions with the citrate method since we have buffered the citrate solutions. Several specimens of phenolsulphonephthalein, the intravenous and intramuscular use of which was followed by reactions, had a $P_H$ 5.0. We have had no reactions since with phthalein solutions properly buffered. We have observed also in a series of twelve cases tested with a dye having a concentration of $P_H$ 5.0 and later with a dye with a $P_H$ 6.95 that from 10 to 40 per cent. more of the properly buffered dye is eliminated in two hours than is the acid type in the same time. We believe that the hydrogen-ion concentration of chemicals or drugs is an important feature in their absorption and elimination both when injected or applied to mucous membranes of the human body.

Several preparations of salvarsan, when prepared according to the directions of the manufacturers, ranged in $P_H$ from 11.53 to 11.99. Silber-salvarsan, $P_H$ 10.71; Neo-salvarsan, $P_H$ 8.15 to 9.22.

A long list of sera, vaccines, and antitoxins prepared by the New York State Department of Health approximated the $P_H$ of the blood except tetanus antitoxin which had a $P_H$ 8.60 and anti-pneumococcus serum, type I, $P_H$ 8.10. Triple pneumococcus vaccine, U. S. Army Medical School, $P_H$ 8.95. These might be sufficiently alkaline to produce local reactions.

All of the common drugs used for hypodermatic medication, both in tablet and ampoule form, were tested. Liquid digitalis and strophanthus preparations were found to have a high concentration, as were preparations made from the pituitary body and suprarenal bodies. Novocaine becomes acid on standing. We found much of the medication sold in ampoule form much more acid than the body tissues or fluids. We believe this to be the main cause of sore arms after the subcutaneous injections of such drugs.

117 (1864)

## On the mechanism by which antigen is removed from the circulation.

By **GEORGE M. MACKENZIE** and **EMILY L. FRUHBAUER.**

[*From the Department of Medicine, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital, New York City.*]

In studying the rate of disappearance of horse serum and the curves for circulating precipitin in a group of serum-treated patients it was noted that individuals who have severe serum disease are good precipitin formers and that at the time the precipitin in the circulation reaches the crest of its curve or soon thereafter the precipitinogen rapidly disappears from the blood stream. On the other hand in those individuals who after a first administration of foreign serum, show very mild or no symptoms of serum disease little or no precipitin is demonstrable in the patient's serum and the precipitinogen persists in the circulation for a long period. Intermediate types were also encountered. From these results it seemed at least plausible to assume that an important factor in determining the rate of disappearance of the foreign serum from the circulation was an intravascular union of antibody and antigen. That such an assumption is erroneous seems probable from the following experiments:

In one series of experiments 12 previously immunized rabbits were injected intravenously with amounts of horse serum (3.00 c.c. or 6.00 c.c.) comparable to the amounts used therapeutically in the group of patients studied. The animals were then bled every second or every third day and the precipitin and precipitinogen in the serum titrated. Six of the animals had a high titer (1:20,000 or higher) of precipitin in the circulation at the time of reinjection, 2 had a moderately high titer, 1 had only traces of precipitin, and 3 had no circulating precipitin. Two of the 3 rabbits with no circulating precipitin had been immunized 10 months previously and at that time had developed a high titer of precipitin which had entirely disappeared before the reinjection. Presumably such previously immunized rabbits which had shown themselves to be good precipitin formers would form antibody in excess earlier than upon first immunization and would therefore dispose of injected antigen earlier than upon first immunization and earlier than fresh rabbits injected with the same amounts of antigen.

A control series of 11 normal rabbits was injected with the amounts of horse serum used in the previously immunized rabbits; precipitin and precipitinogen determinations were made similarly on the controls every second or third day. Of the rabbits receiving 3.00 c.c. of horse serum the time of disappearance in those previously immunized varied from 6 to 17 days with an average of 11.2 days and in the unimmunized controls the variation was 14 to 17 days with an average of 15.5 days. In the rabbits receiving 6.00 c.c. of horse serum the figures were: Immunized, variation 1 to 37 days, average 13.7 days. Unimmunized, variation 6 to 21 days; average 16.3 days. The difference, while in favor of the immunized animals, certainly falls short of theoretical expectations.

Following these preliminary and somewhat inconclusive observations an attempt was made to determine the rôle of intravascular union of antigen and antibody by a different experimental procedure. Normal rabbits were injected intravenously with 6.00 c.c. of horse serum and then twice every day given large amounts of high titer anti-horse rabbit serum intravenously. Precipitin and precipitinogen determinations were made daily during the period of the experiment. No conclusive evidence of accelerated disappearance of antigen was observed. One rabbit which received 97 c.c. of potent (1:20,000 to 1:500,000) anti-serum during the 48 hours following the injection of 6.00 c.c. of horse serum had the foreign serum in the circulation for 7 days. Another rabbit receiving 126 c.c. of potent (1:20,000 to 1:500,000) anti-serum during the 55 hours following the injection of 6.00 c.c. of horse serum continued to have the foreign serum in the circulation for 9 days after the antigen injection. While the rate of disappearance of antigen in these two rabbits is below the average for normal rabbits injected only with horse serum the rate is within the limits of variation of the control rabbits. If such a flooding of the circulation with specific anti-serum produces little or no acceleration of the disappearance of antigen it seems justifiable to assume that intravascular union plays an unimportant rôle in the mechanism for removal of foreign serum from the circulation, and therefore that the cellular phase is of predominating significance.

It is also evident from the results on the whole group of 28 rabbits studied that individual variation extends over a wide range both in ability to form antibody and in the rate at which foreign serum is removed from the circulation.

### 118 (1865)

## The effect of various proteins on streptolysin production.

By **FRANKLIN A. STEVENS** and **CLIFFORD LAMAR.**

[*From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.*]

Variations in streptolysin production in horse and rabbit serum media were noted in a previous publication. In horse serum hemolysin was produced in titratable quantities later in the growth of cultures than in rabbit serum broth but the maximum concentration reached was greater. Unless glucose were present the curve of lysin production corresponded closely to that of growth since hemolysin was found in greater concentration during the period in which the bacteria were multiplying most rapidly. On account of these differences which were characteristic of these sera an attempt was made to discover the responsible factors. The albumen and globulin ratio was modified so that the horse serum contained the same proportions of horse-serum albumen and globulin as were found in rabbit serum, and rabbit serum the same percentages as were present in horse serum. Flasks prepared with 20 per cent. of these modified sera in plain infusion with 0.7 per cent. NaCl, were seeded with equal quantities of a 16-hour culture of hemolytic streptococcus in 20 per cent. horse serum broth. Hemolysin titrations were then made at intervals of an hour with a suspension of horse corpuscles in physiological salt.

The percentages of albumen and globulin in normal rabbit and horse sera were determined by fractioning the diluted serum with ammonium sulfate. The analyses in parts per hundred were as follows:

| | Albumen. | Globulin. |
|---|---|---|
| Horse | 2.6 | 3.5 |
| Rabbit | 3.41 | 2.27 |

Sterile albumen and globulin were obtained respectively from horse and rabbit sera under sterile conditions. The albumen was precipitated with ammonium sulfate. The globulin was prepared by saturating diluted rabbit serum with $CO_2$ and so did not represent the true globulin fraction of the serum.

The horse serum was diluted until it contained 2.27 grams of globulin per hundred c.c. and sufficient horse-serum albumen added to bring the percentage of albumen to 3.41 grams per cent. Rabbit serum was diluted and rabbit-serum globulin added so that the percentages of albumen and globulin were the same as those of horse serum. Flasks of 20 per cent. media were prepared with the modified sera. When growth and streptolysin estimation were made with growing cultures in these media it was found that the reversed ratio of albumen to globulin had no effect on the type of curve obtained. The curves in the modified sera were similar to those in normal serum media. Hence it appears that albumen and globulin do not enter into any of the peculiarities of growth exhibited by hemolytic streptococcus. This fact was further substantiated by a study of the proteolytic enzyme of these bacteria, because the enzyme acted similar to erepsin and did not digest serum albumen and globulin. Furthermore the addition of large amounts of albumen and globulin did not modify the curves.

Growth and hemolysin were next studied in peptone. Two preparations of peptone were used. These were similar except one was partially hydrolyzed and the other broken until further digestion with active trypsin gave no increase in amino nitrogen. The peptone was boiled, filtered through a 10-pound filter and added to beef infusion. Analyses of the media prepared with these peptones after autoclaving were as follows:

| | Total N. Grams per 100. | Amino N. Per Cent. of Total N. |
|---|---|---|
| 1 | 0.27 | 14.9 |
| 2 | 0.28 | 41.8 |

When streptococci which had not been animal-passed were grown in flasks of these media so that comparisons of growth and hemolysin could be made, the growth was more luxuriant, the lag shorter and the hemolysin stronger in the peptone which had been only partially hydrolyzed. This suggests that causes for variations in growth are to be sought in the partially split proteins of the blood serum.

### 119 (1866)

## The bicarbonate and chloride content of the blood in certain cases of persistent vomiting.

By **H. A. MURRAY, JR.** (by invitation).

[*From the Presbyterian Hospital, New York City.*]

These researches were instigated by an interest in the phenomenon of tetany, particularly in that form known as gastric tetany. Very little work has been done in this field on human subjects, none recently; and it seems that no blood analyses have been published. Whatever work was done was not convincing and the old hypotheses such as the dehydration and mechanical theories once offered as a result of clinical studies to explain the condition must be discarded as untenable.

There has been some successful experimental work on dogs, however, in which tetany was produced by obstructing the pylorus and in which various disturbances in the salts of the blood were recorded.

To summarize: McCann[1] (1918) was the one to discover that after pyloric closure there was a rise in the combined carbon dioxide. This was confirmed by MacCallum[2] (1920) and in the surgical laboratories of this college (Hastings[3] and Murray 1921). MacCallum and ourselves also found a markedly diminished chloride content with normal values for calcium. In our laboratory, contrary to expectations, it was found that the H-ion concentration was only slightly and what we considered insignificantly raised. Finally, Dr. Greenwald, who was good enough to analyze specimens from three of our dogs, showed that there was no consistent change in the percentage of sodium.

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[1] McCann, W. S. *J. Biol. Chem.*, 1918, xxxv, 553.

[2] MacCallum, W. G., Lintz, J., Vermilye, H. N., Leggett, T. H., and Boas, E. *Bull. Johns Hopkins Hosp.*, 1920, xxxi, 1.

[3] Hastings, A. B., Murray, C. D., and Murray, H. A. *J. Biol. Chem.*, 1921, xlvi, 223.

The analyses to be reported, indicate that the changes noted in dogs may occur in humans as well.

Subjects with a carbon-dioxide tension of 70 vols. per cent. or over were considered abnormal and included in the group. In the chloride estimations anything below 5.5 grams per liter for plasma or 4.3 grams per liter for whole blood was deemed pathological.

In all, we studied seven cases; three of them had obstructions at or near the pylorus from ulcer, and two from cancer; one man had subacute gastric dilatation following appendectomy, and the seventh member of the group had an annular carcinoma of the lower jejunum. All cases were associated with inordinate vomiting, four days to two months in duration. In a few cases gastric lavage was practised, a procedure which probably aggravated the condition.

The abnormal values varied from 70 to 107 vols. per cent. In the three cases which showed tetany, the highest figures were respectively 103, 104 and 107 vols. per cent. The chloride values varied from 4.5 to 2.2 for whole blood and 5.1 to 3.7 for plasma. In several cases there was an accompanying rise of urea.

In two cases, dilute solutions of hydrochloric acid were given intravenously with a resultant decrease in the $CO_2$ tension. In the last case, 500 c.c. of 0.1 *N* HCl was combined with 500 c.c. of physiological salt solution as an infusion without apparent deleterious effects.

These findings indicate then that persistent vomiting, in all our cases the result of high obstruction of the alimentary canal, will result in an increased bicarbonate and decreased chloride content of the blood. This is undoubtedly due to the loss of hydrochloric acid from the stomach.

The cause of the tetany is not known definitely. Dr. Van Slyke[4] in a recent article stated that uncompensated alkalosis was a cause of tetany. Some of our findings suggest that this is correct. For instance, it was observed that tetany occurred in the three cases in which the $CO_2$ tension was highest. However, our results with dogs do not confirm this hypothesis. Dr. Hastings made the $P_H$ determinations by the gas chain method, using all the accepted refinements. He found that it was normal until just before death, when the usual sudden ante-mortem drop occurred, proving that in this condition, death at least, is not due to an intoxication by hydroxyl ions. Moreover, as far as I know, there are no fundamental experiments to prove that nerve tissue is hyperirritable in the more alkaline solutions. Thus, since we have no direct proof we are not prepared, yet, to say that alkalosis per se, is the cause. There are other ions to be considered besides the hydrogen ion, which may affect nerve irritability. Dr. Loeb[5] has pointed out the importance of the monovalent/divalent kation ratio or more specifically the Na/Ca ratio, and has shown differences in the anions as well. In this case, the sodium-calcium ratio seems to be normal but there is a marked disturbance in the anions which may be the important factor.

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[4] Van Slyke, D. D. *J. Biol. Chem.*, 1921, xlviii, 153.

We are investigating further into the problem, and hope to be able to determine by estimating the hydrogen-ion concentration and the carbon dioxide content in the same sample of blood whether we are dealing with a condition of compensated or uncompensated alkalosis.

### 120 (1867)

## Hen-feathering induced in the male fowl by feeding thyroid.

By HARRY BEAL TORREY and BENJAMIN HORNING.

[*From the Department of Zoölogy, University of Oregon, Eugene, Oregon.*]

It is already well known to the members of this Society that the males of certain breed of fowls—notably Sebright bantams and Campines—are feathered so like the females as to be in this respect practically indistinguishable from them; and that such hen-feathered males, following castration (especially in early life), develop plumage of the usual male type.

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[5] Loeb, J., and Ewald, W. F. *J. Biol. Chem.*, 1916, xxv, 377.

This appearance of cock-feathering in normally hen-feathered males after castration is now familiar to us. It is the purpose of this paper to call attention to experiments that have led to a similar transformation but in just the opposite direction, namely, to the appearance of hen-feathering in cock-feathered males of ordinary breeds.

We had begun a series of preliminary experiments upon the physiological correlations of certain of the ductless glands. Eighty Rhode Island Red chicks were under observation. They were of the same hatch. They had been divided into four lots of twenty each.

All the birds in two of the lots, both males and females, had been castrated between two and four weeks after hatching. Those in the other two lots were unaltered.

Four weeks after hatching, one lot of castrated birds and one lot of normal birds had begun to receive daily doses of dried thyroid (Armour and Company, containing 0.2 per cent. I) by mouth. The initial individual dose was 50 mg. It was increased from time to time. At the end of fifteen weeks it had become 330 mg.—a dose the birds were able to take without any disturbance of their normal health.

There is a striking difference between the sexes of this breed of fowls with regard to the time at which the tail coverts make their appearance. These feathers began to show themselves in some of our birds six weeks after hatching. Five weeks later, they were well developed on the normal females, in both thyroid and control lots. But they had not yet appeared on the control males, either normal or castrated. This absence of tail coverts provided a ready recognition mark of the male. Less conspicuous at this time, but nevertheless unmistakably present on the neck, were the hackles characteristic of the male bird only.

In sharp contrast with these hackled and tailless males were the thyroid-fed normal males. Though the latter were upstanding birds with well-developed comb and wattles, and unquestionably male in carriage and instincts, they were as unquestionably female in plumage, owing to the absence of hackles and the presence of well-developed tail coverts. All doubt as to the sex of these birds was removed when they were killed four weeks later. At this time the control males were typically marked by long hackles, saddle feathers and definite sickles. The thyroid-fed males resembled females in each of these respects. In behavior, comb, wattles and spurs, they were male, in feathering female.

It was clear that the addition of thyroid to the diet of unaltered males was somehow responsible for this condition. Turning to the castrated birds that had been fed thyroid, it was equally clear that the gonad was a necessary factor in the result. For not a single castrated bird of either sex showed the least evidence of thyroid feeding in any of the characters previously enumerated. The thyroid-fed castrated females like their castrated controls, approximated males in feathering. The thyroid-fed castrated males exhibited the ultra male plumage characteristic of capons.

The facts thus far considered relating to the secondary sex characters are arranged for convenience in the accompanying table.

| | Plumage. | Comb and Wattles. |
|---|---|---|
| Normal male | Male | Male |
| Normal female | Male | Female |
| Normal and thyroid male | FEMALE | Male |
| Normal and thyroid female | Female | Female |
| Castrated male | Male | Female |
| Castrated female | Male | Female |
| Castrated and thyroid male | Male | Female |
| Castrated and thyroid female | Male | Female |

Males with hen-feathering induced by thyroid feeding are indicated by capitals. All normal males thus fed were hen-feathered without exception. In two instances, however, sickle feathers began to appear among the tail coverts toward the end of the thyroid-feeding period, suggesting a certain degree of escape from the influence of the thyroid. This was possibly connected with the fact that the birds were then getting less than the maximum dosage of thyroid compatible with their health.

Search for the portion of the gonad through which thyroid feeding achieved its effect led at once to the lutear cells originally demonstrated by Boring and Pearl[1] in the ovary of the hen and later in the testis of the hen-feathered Sebright, by Boring and Morgan.[2] The latter stated their belief that the secretion of these cells suppresses in the hen and in the Sebright male the characteristic cock-feathering.

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[1] *Anat. Rec.*, 1917, xiii, 253.

[2] *Jour. Gen. Physiol.*, 1919, i, 127.

This view seems highly probable, although not, to our knowledge, completely demonstrated as yet. However, if it could be shown that as a consequence of thyroid feeding the lutear interstitial tissue had increased in our hen-feathered male birds, the fact would contribute strong support from a new direction. Our preparations, however, indicate no such hypertrophy. There seems to be little doubt of the complete or nearly complete absence of lutear cells from the normal testis of R. I. R. cockerels at the age of ten to fifteen weeks. The same appears to be true of the thyroid-fed birds, but we are not so confident of the facts in this case because of poor fixation of the tissues. New preparations from experiments now in progress will probably clarify the situation.

The following series, however, adds some significance to the lutear tissue in the present connection.

1. In R. I. R. cockerels, ten to fifteen weeks of age, lutear cells appear to be entirely lacking, and these birds fail entirely to to develop tail coverts of the female type.

2. In White Leghorn cockerels of the same age, lutear cells are present and tail coverts of the female type are present also. In W. L. adult males, however, these cells are lacking, as in adult R. I. R. and correlated with their absence, the full male plumage is present.

3. In Sebright males, hen-feathering and lutear cells are present together in young cockerel and in adult as well.

R. I. R. males do not pass through a juvenal plumage, at least so far as tail coverts are concerned. This is a rather exceptional fact, contrasting sharply with what we have found to be true of the White Leghorn cockerel, and the Sebright, both cockerel and adult. Juvenal plumage would thus appear to be determined, not by age, but by cells whose presence, regardless of age or of sex, leads always to plumage of one type. According to this view, juvenal plumage is female plumage, and female, juvenal—or neither, as in the Sebright adult male. This problem has large implications, consideration of which would carry us outside the proper limits of this paper.

If the appearance of hen-feathering in normal males as a consequence of thyroid feeding is dependent on the presence of lutear cells, it remains to consider the nature of the relation between thyroid and lutear tissue. The former might lead to an augmentation either of the mass or the activity of the latter, or both. There is no evidence as yet of an increase in the mass or number of lutear cells in the testis of thyroid-fed males. The second alternative, for which direct evidence is far less easy to obtain, may yet prove to be the correct one.

### 121 (1868)

## The relation of the diffusion constant to mountain sickness.

By **GEORGE HARROP** (by invitation).

[*From the Presbyterian Hospital, New York City.*]

In connection with the recent physiological expedition to Peru, a series of determinations were made of the gas diffusion constant by the CO method described by Dr. Marie Krogh.[1] A series of preliminary determinations made at sea level agreed in the limits of error (5 per cent.) with those made at Cerro de Pasco, at 14,300 feet. It was found that the severity of the symptoms of mountain sickness, or seroche, exhibited by the eight members of the party were in direct proportion to the value found for their diffusion constants, those having values of over 40 suffering very little, or practically not at all.

TABLE A.

Determination of Diffusion Constant—CO Method.

Cerro de Pasco, Dec. 27–Jan. 10, '22—14,300 Feet.

| Subject. | K. | $D_{O_2}$. | Clinical Phenomena. |
|---|---|---|---|
| H....... | 5.50 | 25.4 | Severe seroche, 3 days, symptoms chiefly nervous. |
| Bo...... | 6.03 | 31.8 | Severe seroche, 6 days, symptoms gastric and nervous. |
| Ba...... | 8.44 | 36.0 | Moderately severe seroche, 2 days in bed. |
| Bi....... | 9.3 | 38.3 | Moderately severe seroche, 2 days in bed. |
| D....... | 5.91 | 41.5 | Practically symptom free throughout stay. |
| R....... | 6.85 | 42.9 | Some headache; otherwise symptom free. |
| F....... | 6.01 | 43.8 | Practically symptom free. |
| M....... | 9.80 | 45.6 | No effects from seroche. |

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[1] *Jour. of Physiol.*, 1915, xlix, 271.

TABLE B.

DETERMINATIONS ON AMERICAN RESIDENTS AT CERRO—14,300 FEET.

| Subject. | K. | $D_{O_2}$. | |
|---|---|---|---|
| P....... | 7.86 | 43.4 | All of these persons have lived two or more years at high altitudes carrying on their work, free from symptoms. |
| McL..... | 9.76 | 44.9 | |
| Cu...... | 7.85 | 44.7 | |
| Co...... | 11.37 | 41.5 | |
| R....... | 12.22 | 65.3 | |

A further series on five acclimatized persons, none of whom had ever suffered from seroche, all gave values for the diffusion constant for oxygen ($D_{O_2}$) above 40.

### 122 (1869)

## An undescribed relation of the suprarenals to ovulation.

By **OSCAR RIDDLE.**

*[From the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.]*

The observations described here make it extremely probable that the suprarenal glands regularly and greatly enlarge in close relation to the time of liberation of ova from the ovary. The maximum size seems to be attained in the 44-hour interval between the ovulation of the first and second ova—which together constitute a definite period of ovarian activity in the pigeon. The early stages of the suprarenal enlargement are coincident with the 4 to 5 days of extremely rapid growth[1] which these ova undergo immediately before their expulsion from the ovary. Knowledge of the exact time (within an hour) of ovulation in the pigeon has made this result possible. An enlargement of the oviduct also occurs quite parallel with that of the suprarenals (both facts shown by curves and tables). That an hypertrophy of the suprarenals occurs in some sort of relation to the menstruation, pregnancy and lactation of mammals has of course been described; so far as we are aware the nexus with ovulation has been overlooked.

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[1] Riddle, O., *Amer. Jour. Physiol.*, 1916, xli, 387.

Before undertaking the present study we had learned that birds dead of tuberculosis, or from the presence of round-worms (Ascaridia), usually show enlarged suprarenals. The normal size of each suprarenal had been found to lie between 0.006–0.009 gram; those dead of tuberculosis weighed as much as 0.051 g. and the Ascaridia-infested were almost equally enlarged. Enlargement of these glands doubtless occurs under many other infections. Forty-three females with fully known reproductive history were taken for this study; they were killed at several intervals with reference to ovulation; they were placed in one of two groups according to whether they showed or failed to show round-worms or tuberculosis. The data obtained from the healthy birds show that in nearly (not quite) all cases the supra-

CURVE SHOWING DATA FOR HEALTHY PIGEONS.

The 44-hour ovulation period (shown between the two vertical lines) occupies for convenience only one half the space it should occupy. All other time intervals are properly spaced. The ordinates represent weight. The number of birds concerned at each point in the curve—from left to right—follows: 3, 4, 6, 3, 1, 2, 3, 1

DATA FOR SIZE OF SUPRARENALS IN RELATION TO THE PERIOD OF OVULATION.

| Period with Reference to Ovulation. | No. of Birds. | Ave. Weight (Grams). | | |
|---|---|---|---|---|
| | | Body. | Oviduct. | Adrenals. |
| Healthy Common Pigeons. | | | | |
| 96 + hrs. before | (3) | 312 | 0.883 | .0076 |
| 29–53 hrs. before | (4) | 351 | 4.957 | .0148 |
| 3–22 hrs. before | (6) | 360 | 10.356 | .0207 |
| *Mid-Ovulation* | (3) | *355* | *8.781* | *.0219* |
| 2–3 hrs. after | (1) | 373 | 9.483 | .0197 |
| 20 hrs. after | (2) | 344 | 7.886 | .0136 |
| 45–73 hrs. after | (3) | 343 | 2.987 | .0164[1] |
| 96 + hrs. after | (1) | 338 | 0.883 | .0108 |
| Common Pigeons bearing Ascaridia or Tuberculosis. | | | | |
| 96 + hrs. before | (2) | 314 | 1.499 | .0097 |
| 48–72 hrs. before | (3) | 348 | 3.725 | .0166 |
| 24 hrs. before | (3) | 331 | 6.823 | .0096 |
| 3 hrs. before | (1) | 323 | 8.382 | .0119 |
| *Mid-Ovulation* | (7) | *336* | *8.907* | *.0122* |
| 1–3 hrs. after | (2) | 366 | 8.649 | .0141 |
| 20 hrs. after | (1) | 340 | 7.828 | .0138 |
| 50 hrs. after | (1) | 340 | 4.708 | .0137 |
| 96 + hrs. after | (2) | 314 | 1.499 | .0097 |

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[1] For two of the three the average is 0.0095.

renals were enlarged, and most enlarged in the middle of the ovulation period. The diseased birds fail to show this relation; and, though these glands are larger than the normals in the periods most removed from ovulation, they do not show a comparable enlargement during ovulation.

The appearance of the hypertrophied glands in the normal birds is otherwise wholly normal. Their appearance certainly suggests an increased activity of the glands during this period. Sections of the gland have not been made and we do not know whether both parts share in the hypertrophy. If increased secretion occurs, it doubtless has an important bearing upon the general physiology of the gland; and upon the effects which "reproductive overwork" (Whitman, Riddle) has been found to produce on the egg size and offspring of pigeons; for, a bird made to produce as many as 30 or 40 pairs of eggs per year would thus be almost continuously subjected to a hypersecretion of the suprarenals.

## 123 (1870)

## Studies in the physiology of vitamins. III. A comparison of the effects of feeding extracts of muscle and yeast respectively.

By **GEORGE R. COWGILL.**

[*From the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Conn.*]

In a previous communication[1] experiments were reported wherein it was shown that the feeding of extracts of rice polishings, wheat embryo, and navy bean to dogs which had been fed on a diet lacking vitamin-B resulted in a recovery of appetite which lasted for varying periods. Vitamin-B was suggested as the appetite-promoting factor in the preparations used. The present report concerns control experiments in which an extract lacking this factor was tested.

Commercial Liebig's extract of beef muscle and the extract of yeast vitamin as prepared by the Harris Laboratories were used for these experiments. Tests were made using the Liebig

[1] Cowgill, PROCEEDINGS SOC. EXP. BIOL. AND MED., 1921, xviii, 290–291.

extract administered either with single large doses or with large doses repeated daily for as many as fourteen (14) days, the material being introduced by stomach sound in order to eliminate the taste factor. In another series of experiments the meat extract was mixed with the food which had been refused. The meat extract did not restore the appetite to such animals. On the other hand relatively small amounts of the yeast extract given by stomach produced a prompt recovery of appetite which lasted for from four (4) to nineteen (19) days depending on the amount administered.

Liebig's extract in doses such as were employed in these experiments promotes the flow of gastric juice in normal animals.[2] This fact, together with our own observation that products containing vitamin-B do not promote the flow of saliva, pancreatic juice, or bile,[3] suggests that the recovery of the desire to eat in our animals is not to be ascribed to an increased flow of gastric juice.

124 (1871)

## Studies in the physiology of vitamins. IV. Parenteral administration of products containing vitamin-B—mammalian experiments.

By GEORGE R. COWGILL.

[*From the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Conn.*]

That relief from the symptoms due to a lack of vitamin-B in pigeons may be brought about in a very short time by intramuscular injection of products containing this vitamin has been shown by many investigators. Studies of parenteral administrations of vitamin-B to mammals do not appear to have been made. In the course of our studies into the physiology of this vitamin, using dogs as experimental animals, the protein-free concentrate of vitamin-B from yeast as prepared by the Harris Laboratories was used, sufficient amounts of this product being generously furnished by Dr. I. Harris.

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[2] Pawlow, "The Digestive Glands," 1902, 96.

[3] Cowgill, PROCEEDINGS SOC. EXP. BIOL. AND MED., 1921, xviii, 148–149; ibid., 290.

Intravenous injections of a neutral aqueous solution of this product into dogs showing severe nervous symptoms (clonic spasms, etc.) due to a lack of vitamin-B resulted in a complete cessation of such symptoms, in one instance within a half hour, and in another case within three hours after the injection. In a third instance the relief from symptoms occurred after a longer period. An intravenous injection of the vitamin-containing product was also made into an animal which had lost its appetite after subsisting on the vitamin-free food for a period of days but which showed none of the nervous symptoms characteristic of vitamin-B deficiency. The injection was followed by a complete recovery of appetite which lasted six (6) days.

Intraperitoneal injections were also made. Such an injection, while bringing about relief from nervous symptoms in all cases, did not prevent death from supervening from ten (10) to twelve (12) hours later in those instances where large amounts of material were injected. A control injection into a normal animal resulted in phenomena indicating that the fatal outcome in the instances cited was probably due to the too sudden introduction of large amounts of material and the effect of this procedure on the tissues within the peritoneal cavity.

The effect of subcutaneous injections into such animals will be studied shortly.

### 125 (1872)

## The conductance of unicellular organisms.

By S. C. BROOKS.[1]

[*From the Division of Pharmacology, Hygienic Laboratory, U. S. Public Health Service, Washington, D. C.*]

The electrical conductance of bacteria (*B. coli* and *B. butyricus*) unicellular algæ (*Chlorella* sp.) yeasts (*Saccharomyces* sp.) and mammalian red-blood cells has been studied by a method which yields figures for the gross conductance dependable within 1/10 per cent. Polarization capacity was compensated for by variable condensers in parallel with the variable resistance. While most of the studies here reported were made on the alga, *Chlorella*, there is apparently no fundamental difference between this and the other organisms.

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[1] Approved for publication by the Surgeon General.

The difference between the conductance of the suspending fluid and that of the suspension of cells is expressed in per cent. of the former and called the net conductance. This will always be negative in sign, because living cells are poor conductors of electricity. Rather large variations in the net conductance are produced by factors, such as irregular distribution of cells in the suspension, which it is impracticable to eliminate and which may affect the net conductance of any one sample by several per cent. For this reason enough samples were used in each experiment to make the error of the mean less than three per cent.

It is possible to calculate the limits between which the observed net conductance should fall, the relative conductance of the cells themselves and their volume concentration being known.

If $r$ is the resistance of the suspending fluid and $kr$ that of the cells and $n$ the concentration of the cells in volume per cent., then the total resistance should lie between

$$10r\frac{\sqrt{n}(k-1)+10}{(10\sqrt{n}-n)(k-1)+100}$$

and

$$r\left(1+\frac{n(k-1)}{10\sqrt{n}(1-k)+100k}\right).$$

These formulas give curves convex to the axis of concentrations when they are plotted against resistances as ordinates. But when the cells form less than about 65 per cent. of the total volume the observed curves are nearly linear, possibly because of variations in the relative extent to which the current lines are able to evade the more highly resistant cell material. It is fortunate that this is so, since in case the volume of cells varies during the course of an experiment (*e.g.*, because of osmotic changes) a linear correction may be introduced. Different organisms and even different lots of the same organism differ considerably in their net conductance at any given concentration.

The relation between the conductance of the suspending fluid and the net conductance of the suspension was somewhat unexpected. Perrier's artificial sea-water[1] was diluted to 2, 4 and 8 volumes with distilled water. *Chlorella* which had been grown in a solution somewhat more dilute, was transferred successively to increasing concentrations of this artificial sea-water and the net conductance determined several times in each case until upon further renewal of the solution between each determination no further change occurred. For example, in changing from 1/8 to 1/4 strength sea-water, the net conductance changed from 28.9 to 27.2 per cent. Corrected for the decrease in the volume of cells, the figures would be 28.9 and 28.3 per cent. The change in net conductance is within the limits of error of the method. This shows that if by any chance the conductivity of the surrounding medium is changed during the course of an experiment only the net conductivity in per cent. will normally remain constant. Expressed in any other way the results might easily be misleading. In the above case the net resistance expressed in ohms instead of remaining constant fell from 80 to 45 ohms approximately.

It is also of interest to note that the net conductance of dead cells is relatively greater in those organisms which normally encounter a fluctuating environment. The ratio of the net conductances of dead and living cells is about as follows: red blood cells .45, yeast .65, bacteria .70, Chlorella .80–.90. This fact suggests that the cell walls (or at least some non-living structure), help to protect such cells from extreme fluctuations in electrolyte concentrations, insofar as the changes inside the cell walls would be retarded and therefore less abrupt. Multicellular forms such as *Laminaria* need such protection only at the outer surfaces of the superficial cells, and it is therefore not surprising that such tissues have when dead only about one tenth the electrical resistance that they have when living.

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[1] Perrier, E., *Comptes Rendus*, 1890, cx, 1076.

### 126 (1873)

## The hydrogen-ion concentrations of joint exudates in acute arthritis.

By **RALPH H. BOOTS** and **GLENN E. CULLEN.**

[*From the Hospital of the Rockefeller Institute for Medical Research, New York City.*]

The hydrogen-ion concentrations of exudates aspirated from joints of patients ill with acute rheumatic fever and other forms of arthritis were determined. This was done: (1) to compare the reactions of the exudates of the various forms of arthritis; (2) to determine if an acidity existed in inflamed joints of acute rheumatic fever patients sufficient to permit the liberation of free salicylic acid following salicylate therapy.

Salicylic acid can not exist as such in alkaline solutions; and its salts have not been shown to have bactericidal power in low concentrations. Although the acid can not exist in normal blood and tissues, their reactions being slightly alkaline, it has been suggested for a number of years that its liberation might occur in the inflamed tissues of patients with acute rheumatic fever; these tissues were supposed to have been under considerably increased $CO_2$ tension. The bactericidal action of this liberated salicylic acid could explain the seemingly specific action of the salicylates on the arthritis of acute rheumatic fever.

Hanzlik[1] examined exudates from inflamed joints of acute rheumatic fever patients directly for the presence of salicylic acid. The results showed none to be present; but the author offers the criticism that no precaution was taken to prevent the escape of $CO_2$.

In our work, the hydrogen-ion concentrations of all of the exudates were determined colorimetrically at room temperature and corrected to 38° C. by a method recently described by Cullen.[2] If sufficient fluid was obtained from a joint, the determination was also made electrometrically. With both methods, in order to prevent the escape of $CO_2$, the fluid was not allowed to come into contact with the air.

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[1] *Jour. Pharm. and Exp. Therapeutics*, 1917, ix, 217.

[2] *Jour. Biol. Chem.*, 1922, l, 17.

*Results.*—The reactions of 16 joint exudates from patients with acute rheumatic fever were all slightly alkaline; their hydrogen-ion concentration varied from $P_H$ 7.2 to 7.38. Seven exudates from patients with chronic arthritis varied in $P_H$ from 7.27 to 7.4. An exudate aspirated from a knee infected with *Staphylococcus aureus* had a $P_H$ of 6.63 and that from a knee infected with *Streptococcus hæmolyticus* was also acid, having a $P_H$ of 6.14.

Since a definitely acid medium is necessary for the liberation of salicylic acid, and since all of the joint exudates from acute rheumatic fever patients were slightly alkaline, no free salicylic acid can exist in these joint fluids following the administration of salicylates.

## 127 (1874)

## The selective bactericidal effect of acid fuchsin and sodium chloride.

By **JOHN W. CHURCHMAN.**

[*From the Department of Hygiene, Cornell University Medical School, New York City.*]

In 1912 it was found that if bacteria be stained with gentian violet and planted on plain agar a sharp selective activity of the dye could be readily demonstrated. All the commoner gram-negative organisms survived even long exposure to the stain, while all the commoner gram-positive spore-bearing aërobes were "killed," even by a relatively short exposure. Even the spores—though not deeply, if at all, stained by gentian violet—were "killed" by exposure to the dye. What was true of gentian violet was found to be true also of other *basic* dyes of the tri-phenyl-methane group.

It is now found that a cleavage in exactly the opposite sense occurs if organisms be exposed to acid fuchsin—an *acid* dye of the tri-phenyl-methane series. Whereas gentian violet kills the gram-positive spore-bearing aërobes and spares the commoner gram-negative bacteria, acid fuchsin spares the former and kills the latter. The experiments on which this statement is based were done with *B. subtilis*, *B. megatherium*, *B. anthracis*, *B. typhosus*, *B. coli communis*, *B. prodigiosus*, *B. pyocyaneus*, *B. proteus vulgaris*. In the case of gentian violet the reaction is evident if the stain is applied to the organisms at room temperature; but in the case of acid fuchsin, while long exposure to the dye at room temperature produces the reaction, a slight increase in temperature (to 45° C.) makes it much sharper and speedier.

In the case of gentian violet it was shown, by a study of the whole bacterial field, that the gentian-violet reaction and the gram reaction ran parallel in a striking way. Not only did the cleavage hold between gram-negative organisms and the commoner *spore-bearing aërobes*; it also held between gram-negative bacteria and the great majority of *non-spore-bearing gram-positive organisms*. That a reverse parallelism holds for acid fuchsin has not been proven. Of the greater susceptibility to this dye of the gram-negative organisms (as compared with the gram-positive *spore bearers*) there is no doubt; it is not established that a similar cleavage holds between gram-negatives and gram-positive *non-spore bearers*. An interesting practical point is the susceptibility of the gram-negative *B. pyocyaneus* to acid fuchsin; this organism is often a great nuisance in open wounds and resists ordinary antiseptics.

What has been said of acid fuchsin is true also of another and wholly unrelated acid dye—ponceau P.R. It is also true of sodium chloride. When the commoner gram-negative organisms are exposed to saturated solutions of any of these three substances at 45° C., they are killed; while gram-positive spore bearers are unaffected under similar conditions. This is the exact reverse of the behavior of these two classes of bacteria toward basic gentian violet.

In the case of gentian violet, the selective effect of the dye may be demonstrated either by staining the organisms and planting them on plain agar or by planting them unstained on agar containing the dye. The gram negatives grow well in the presence of the dye, while the gram positives will not grow at all, even when the stain is present in the media in very small amounts. For this and other reasons the activity of the dye has been referred to as bacteriostatic, since it seems to consist essentially in an inhibition of growth. It is probable that this parallelism between the bacteriostatic and the bactericidal activity of the dye, so clear cut in the case of gentian violet, does not hold in the case of acid fuchsin and sodium chloride. The evidence thus far gathered would indicate that the mechanism by which growth is inhibited may be entirely different from the mechanism by which organisms are killed.

### 128 (1875)

## On the method of macronuclear disintegration during endomixis in Paramecium aurelia.

By **LORANDE LOSS WOODRUFF** and **HOPE SPENCER.**

[*From the Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.*]

In the original studies[1] of the nuclear phenomena involved in endomixis, it was found that macronuclear disintegration, both in *Paramecium aurelia* and *Paramecium caudatum*, was effected by the elimination of spherical chromatin-bodies from the macronucleus, instead of by the transformation of most of the macronucleus into long tangled chromatin-ribbons such as occurs during conjugation in these species. Regarding *Paramecium aurelia*, it was stated that the "differences between the macronuclear changes during conjugation and during the process (endomixis) are only morphological; on the one hand, the macronucleus forms 'wurstförmige Schlingen,' while on the other, the macronucleus eliminates its chromatin by extruding it in the form of spherical bodies." [2]

This contrast proved valid not only in Woodruff's pedigree race[3] (I) of *Paramecium aurelia* in which endomixis was discovered, but also in animals from such diverse sources as Germany and Ohio. Only one cell (*Paramecium aurelia*, I), 4087th generation, December 6, 1913) was observed during endomixis which in any way even suggested ribbon formation and a figure was given of this animal with the legend "An atypical form of macronuclear disintegration, slightly resembling the ribbon-like formation characteristic of conjugation." [4]

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[1] L. L. Woodruff and Rhoda Erdmann, *Journ. Exper. Zoölogy*, 1914, xvii, 425–517. Erdmann and Woodruff, *Journ. Exper. Zoölogy*, 1916, xx, 59–97.

[2] Woodruff and Erdmann, loc. cit., pp. 438–39, and 444; Plate 1, Figs. 9 and 11; Plate 2, Fig. 14; Plate 3, Fig. 32.

[3] Woodruff, *Biological Bulletin*, 1917, xxx, 51–56.

It is therefore interesting to record that the study of animals from this same pedigree culture (I) of *Paramecium aurelia* at about the 8900th generation (November, 1921) showed some cells successfully undergoing endomixis with macronuclear disintegration by ribbon formation and others by chromatin-body formation.

Thus it is clear that although all the data thus far at hand indicate that chromatin-body formation is the typical method of destroying the macronucleus in endomixis, nevertheless under certain unknown conditions the formation of chromatin-ribbons, until now regarded as diagnostic of conjugation, occurs in endomixis. This fact, of course, in nowise narrows the significant and crucial difference between endomixis and conjugation—the absence of synkaryon formation in the former and its presence in the latter.

### 129 (1876)

## Nutritive factors in plant tissues. V. Further observations on the occurrence of vitamin-B.

By **THOMAS B. OSBORNE** and **LAFAYETTE B. MENDEL.**

[*From the Laboratory of the Connecticut Agricultural Experiment Station, and the Sheffield Laboratory of Physiological Chemistry in Yale University, New Haven, Conn.*]

In the course of our studies of the distribution of vitamins in plant products we have collected data regarding a number of important edible foods for which no information in this respect seems to be available at present, with the possible exception of indirect suggestions obtained by other than animal feeding trials. Our experiments, made with rats, supplement numerous earlier ones[1] conducted by the same technique and indicate that *asparagus*,

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[4] Woodruff and Erdmann, loc. cit., Plate 4, Fig. 37.

[1] Osborne, T. B., and Mendel, L. B., *Jour. Biol. Chem.*, 1919, xxxvii, 187; xxxix, 29; 1920, xli, 549; xlii, 465.

*celery*, *dandelion*, *lettuce*, and *parsley* all *contain noteworthy amounts of vitamin-B*. The details of the investigation will be published elsewhere.

### 130 (1877)

## An experiment on the absorption of glucose given by rectum.

By **ROGER S. HUBBARD** and **DAVID C. WILSON**.

[*From the Clifton Springs Sanitarium, New York.*]

For many years it has been accepted by clinicians that glucose given by rectum is absorbed immediately into the blood, and surgeons constantly use such a procedure as a part of their post-operative therapy. Because of the difficulties attending the procedure there are comparatively few controlled experiments on the effect of glucose so administered in the literature. The effect of carbohydrate feeding on acetonuria has been clearly demonstrated by many experiments during the past few years, and it was determined to test the rectal absorption of glucose by studies of the effect produced upon experimental acetonuria.

The subject of the experiment (one of the authors, D. C. W.) was a man 5 ft. $9\frac{1}{4}$ in. tall who weighed 165 pounds, and whose basal metabolism, as measured by the portable Benedict calorimeter was 1700 calories. He received the diet recently discussed by Hubbard and Wright[1] for four days. This diet furnished 2,142 calories—twenty per cent. more than the basal requirement—and consisted of 54 grams of protein, 54 grams of carbohydrate, and 190 grams of fat. Ten per cent. of the calories in this diet are furnished by protein, ten per cent. by carbohydrate, and eighty by fat[2,3]. The total food intake was probably not sufficient for the needs of the subject.

Acetonuria developed gradually as shown in Table I, and on the fourth day of the experiment, before the acetone excretion had reached its highest level, an enema consisting of 300 c.c. of a 5 per cent. glucose solution was given. Table II shows figures for the morning of the day before the enema was given, for the specimen collected just before the enema was given, and for the specimen which corresponded to the period during which the enema was retained. Both tables show that there was a decrease in the concentration of acetone plus acetoacetic acid caused by the glucose, while the $\beta$-hydroxybutyric acid did not show the increase which is usually caused by such diets.[1] The decrease was not as great as that caused by similar amounts of glucose when taken by mouth. Shaffer[2] has recently advocated the theory that the failure of acetoacetic acid to burn completely in the absence of glucose is responsible for the appearance of increased amounts of the acetone bodies in the urine. The observation that the aceto-

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[1] Hubbard, R. S. and Wright, F. R., *J. Biol. Chem.*, 1922, l, 361.

[2] Zeller, H., *Arch. Physiol.*, 1914, p. 213.

[3] Shaffer, P. A., *J. Biol. Chem.*, 1921, xlvii, 449.

TABLE I.

| Date, 1921. | Urine. | | | | | Alv. $CO_2$ mm. |
|---|---|---|---|---|---|---|
| | Vol. c.c. | Acetone. | | $\beta$-hydroxy. | | |
| | | $\frac{\text{mg.}}{100\ \text{c.c.}}$ | gms. | $\frac{\text{mg.}}{100\ \text{c.c.}}$ | gms. | |
| 15–16[3]..... | 910 | 1.1 | 0.010 | 1.7 | 0.015 | 40 |
| 16–17...... | 780 | 3.6 | 0.029 | 2.7 | 0.021 | 36 |
| 17–18...... | 770 | 7.8 | 0.060 | 3.4 | 0.026 | .. |
| 18–19...... | 852 | 25.2 | 0.215 | 11.9 | 0.101 | 32 |
| 19–20...... | 628 | 18.7 | 0.118 | 15.2 | 0.096 | .. |

300 c.c. 5 per cent. glucose at 9:30 A.M. on December 19.

Results of the determinations of the acetone bodies are expressed in terms of acetone. Under acetone the results of the determination of acetone from acetone plus acetoacetic acid are given.

TABLE II.

| Date, 1921. | Time, A.M. | Urine. | | | | |
|---|---|---|---|---|---|---|
| | | Vol. c.c. | Acetone. | | $\beta$-hydroxybutyric Acid. | |
| | | | $\frac{\text{mg.}}{100\ \text{c.c.}}$ | gms. | $\frac{\text{mg.}}{100\ \text{c.c.}}$ | gms. |
| 12/18.... | 8:30 to 11 | 102 | 19.2 | 0.020 | 13.3 | 0.014 |
| 12/19.... | 8:30 to 9:30 | 40 | 13.0 | 0.005 | 5.5 | 0.002 |
| 12/19.... | 9:30 to 12 | 108 | 7.8 | 0.008 | 4.6 | 0.005 |

300 c.c. of 5 per cent. glucose given by rectum at 9:30 A.M. on December 19.

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[1] Hubbard and Wright, *loc. cit.*

[2] Shaffer, *loc. cit.*, p. 433.

[3] Day before the diet was taken.

acetic acid shows a decrease in these experiments, while the β-hydroxybutyric acid only fails to show the "expected increase" seems to be in accordance with this view.

The experiment shows that glucose is absorbed rapidly by rectum, and is taken into the blood in sufficient quantities to decrease the excretion of acetone produced by a diet high in fat.

### 131 (1878)

## Effect of dilution on the precipitation reaction for syphilis proposed by author.

By R. L. KAHN.

[*From Bureau of Laboratories, Michigan Department of Health, Lansing, Michigan.*]

One of the important differences between the precipitation reactions of Meinicke, Sachs and Georgi, Dryer and Ward and that proposed by the author[1,2] is that in the last reaction, the amount of normal salt solution is reduced to a minimum. It was early observed, when adding given amounts of serum and antigen to a series of tubes and subsequently adding increasing amounts of normal salt solution to that series, that the degree of

TABLE SHOWING DELAYING EFFECT OF PHYSIOLOGICAL SALT SOLUTION ON PRECIPITATION REACTION FOR SYPHILIS PROPOSED BY AUTHOR.

| | Tube No. | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Syphilitic serum, c.c. | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Antigen, c.c. | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 | 0.025 |
| Salt solution, c.c. | .... | 0.1 | 0.2 | 0.4 | 0.6 | 0.8 |
| Results after 1 hour incubation in water bath | Marked Precipitation. | Moderate Precipitation. | Doubtful Precipitation. | Negative. | Negative. | Negative. |

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[1] PROC. SOC. EXPER. BIOL. AND MED., 1922, xix, 182. The preparation of antigen is briefly described in this paper. It should be cholesterinized to 0.4 per cent. before diluting with salt solution.

[2] *Arch. Derm. and Syphil.*, 1922, v.

precipitation following incubation is inversely proportional to the amount of salt solution added. The tube, for example, which contains serum and antigen without salt solution may show marked precipitation after 1 hour incubation; the one which contains 0.2 c.c. may show moderate precipitation while the one which contains 0.4 c.c. of salt solution may show no precipitation after the same period of incubation. The above table illustrates this point.

The question presented itself whether the delay in precipitation in those tubes which received salt solution as indicated in the table was due to sodium chloride or the element of dilution. A series of experiments was thereupon carried out employing distilled water, 0.425 per cent. and 2 per cent. sodium chloride solutions and the regular antigen mixture employed in the tests. The results in each case were similar to that obtained with normal salt solution, indicating that the delaying effect is produced by the dilution element and not by the sodium chloride. Of particular interest is the finding that the addition of increasing amounts of antigen exerts the same retarding effect on precipitation as, for example, distilled water or normal salt solution.

### 132 (1879)

## Relation between serum and antigen in precipitation reaction for syphilis proposed by author.

By R. L. KAHN.

[*From Bureau of Laboratories, Michigan Department of Health, Lansing, Mich.*]

The optimum relation between serum and antigen in the precipitation test for syphilis proposed by the author has been observed to be in the neighborhood of 4:1. On the basis of this relation one may employ 0.4, 0.3, 0.2 or 0.1 c.c. of serum with 0.1, 0.075, 0.05 or 0.025 c.c. of antigen respectively with similar results. This relation holds true also with smaller quantities than 0.1 c.c. Thus 0.04 c.c. of serum with 0.01 c.c. of antigen has been found to give results comparable with 0.4 c.c. of serum and 0.1 c.c. of antigen. Increasing the amount of antigen beyond this relation tends to delay precipitation, while the employment of equal amounts of serum and antigen will inhibit the reaction in some cases.

### 133 (1880)

## The use of morphine in connection with serumtherapy of botulism.

By **J. BRONFENBRENNER** and **H. WEISS.**

*From the Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston, Mass.*]

Some time ago[1] we have observed the delay in the rate of absorption of botulinus toxin in the animals subjected to ether anesthesia. Combining ether anesthesia with the specific serum therapy, we have been able to save animals poisoned with botulinus toxin where antitoxin alone failed to do so.

In view of the fact that the application of ether anesthesia in cases of botulinus poisoning in men would be difficult on account of the pronounced respiratory distress present as a predominant symptom in such cases, we have attempted to find a satisfactory substitute for ether anesthesia.

Among various substances thus far tried with various degrees of success, morphine seems to give the best results. Thus, one-quarter of a cubic centimeter of botulinus toxin (125 M.L.D.) given by the mouth causes death of a guinea pig of 250 grams in from 10 to 12 hours. The administration of an excess of antitoxin intracardially does not save the animal if more than three hours have been allowed to elapse between the feeding of the toxin and subsequent injection of antitoxin.

If guinea pigs of 250 grams are similarly fed with 125 M.L.D. of botulinus toxin and if 0.02 gram of morphine is given to them subcutaneously (in 10 per cent. solution) soon after feeding of the toxin, such guinea pigs die in from 26 to 46 hours if not given any antitoxin. Thus 0.02 gram of morphine delays the death from botulinus poisoning and more than doubles the length of the life of the animal. If antitoxin is introduced intracardially into such animals (treated with morphine) the animals can be saved even as late as 24 hours after ingestion of toxin.

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[1] PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 253.

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### 134 (1881)

## The state of aggregation of particles of botulinus toxin.

By J. BRONFENBRENNER and M. J. SCHLESINGER.

[*From the Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston, Mass.*]

The fact that one cubic centimeter of botulinus toxin with only 10 per cent. of solids contains at least as many as $10^{18}$ units of toxin when its hydrogen-ion concentration is adjusted to about $P_H = 4$,[1] suggests that the molecules of this toxin must be very small and of a comparatively simple structure.

On the other hand we have shown[2] that saturation of the toxic filtrate of the culture of *B. botulinus* with $(NH_4)_2SO_4$ precipitates the toxin, thus suggesting either that toxin is adsorbed by the coarser particles of the precipitate, or that, contrary to the above assumption, the matrix of the toxin is more complex and is capable of being salted out by the $(NH_4)_2SO_4$.

In order to determine which is the case a number of experiments were undertaken. While they did not yield as yet any definite answer to the question of the size or the nature of the molecule of botulinus toxin, they disclosed certain interesting, though not as yet correlated facts concerning the properties of this extremely active substance. We feel that these facts are sufficiently interesting to justify our reporting them at this time.

I. Crude toxin kills mice in the dose of $3 \times 10^{-7}$ c.c. If its reaction is adjusted to about $P_H = 4$ (at the temp. of 37° C.) its potency is increased so that the M.L.D. $= 3 \times 10^{-18}$ c.c.[1] If one adds to such an acidified toxin a solution of pepsin (at 37° C.), already 5 minutes after the addition of pepsin the potency of toxin is markedly reduced and after four hours of contact with pepsin the M.L.D. of toxin is reduced to that of the original filtrate (M.L.D. = $3 \times 10^{-7}$ c.c.), but no further reduction of toxicity could be observed during further incubation for 48 hours.

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[1] Bronfenbrenner and Schlesinger, PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 1.

[2] Bronfenbrenner and Schlesinger, PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 254.

That the reduction of toxicity is not due to peptic digestion follows from the fact that toxicity was only partly destroyed and in fact was reduced exactly to the level at which it existed in the crude toxin. Besides, identical reduction of potency was observed when *heated* (80° C.) pepsin was added to the acid toxin. Neither could the reduction of toxicity be ascribed to spontaneous deterioration of toxin, since the control mixture consisting of toxin and acid buffer alone maintained its high toxicity to the end of the experiment (48 hours). On the other hand the failure of pepsin to completely digest the toxin is not due to the possible presence in the toxin of some inhibiting substance, since the activity of pepsin against edestin in the controls proceeded equally well in the presence as in the absence ot the culture filtrate of *B. botulinus.*

Thus the reduction of potency of the acidified toxin in the presence of pepsin is not due to its digestion, but most likely to some physical phenomenon such as ready adsorption (?), which possibly returns the particles of toxin from the state of high degree of dissociation to the more stable state where they exist in a condition of undissociated aggregates.

II. As we have previously stated [1] the crude toxic filtrate of the culture of *B. botulinus* killing mice by the intraperitoneal injection in the amount of $3 \times 10^{-7}$ c.c. can be rendered so potent by acidification that it will kill mice in the dose of $3 \times 10^{-18}$ c.c. However, if this crude filtrate is salted out with $(NH_4)_2SO_4$, the precipitate which contains all of the original toxin in a concentrated state *can no longer* be rendered more potent by acidification.

If the tentative assumption that the toxicity of botulinus toxin may depend on the degree of dispersion of its particles is correct, and if in the original toxin the coarser aggregates may be dispersed by acidification, it seems that precipitation with ammonium sulphate renders the particles uncapable of dispersion.

III. If the changes in the structure of botulinus toxin, brought about by the adjustment of its hydrogen-ion concentration are of the nature of an increase of dispersion of its particles, one might expect that the smaller particles of such a dissociated toxin might dialyze easier than the coarser aggregates of the crude toxin. The experimental evidence indicates the reverse to be the fact. While the crude toxin dialyzes with a comparative ease, the acidified toxin remains quantitatively inside the parchment thimble.

May not this phenomenon be explained on the basis of the theories offered by some physical chemists[3] namely, that while the coarser aggregates of protein carry no charge, the increase in the dispersion resulting upon dilution and especially upon acidification confers the electrical charge on such dissociated particles. In virtue of this charge these smaller particles are adsorbed to the membrane whereas the coarser particles of undissociated substance were able to go through the pores of the membrane.

## 135 (1882)

## The diagnosis of kala-azar by blood culture.

By **CHARLES W. YOUNG** and **HELEN M. VAN SANT** (by invitation)

[*From the Department of Medicine, Peking Union Medical College, Peking, China.*]

The usual method for diagnosis of kala-azar by means of cultures has been from spleen juice obtained by puncture and aspiration with a syringe and needle. Prolonged bleeding-time is fairly constant in advanced kala-azar. The risk of bleeding from the needle wound in the spleen together with the possibility of tearing that organ by a sudden movement of the patient during puncture, makes a safer method for cultural diagnosis desirable.

Meyer and Werner[1] reported in 1914, five successful cultures from a single specimen of blood. Row[2] and Korke[3] each report one. Cornwall and LaFrenais[4] succeeded in seven cases; however,

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[3] Robertson, T., Brailsford. The Physiological Chemistry of the Proteins, 1918, p. 153.

[1] Meyer, M., and Werner, *Deutsch. Med. Wchnschr.*, 1914, xl, 67.

[2] Row, R., *Indian Jour. Med. Res.*, 1914, July (quoted in (4)).

[3] Korke, V., idem.

[4] Cornwall, J. W., and LaFrenais, H. M., *Indian Jour. Med. Res.*, 1915–16, iii, 698.

Knowles[1] in attempting to repeat the work using the methods of Row and of Cornwall and LaFrenais only obtained two positive cultures out of 129 tubes from thirty-four cases. Meyer and Werner, Cornwall and LaFrenais, and Knowles (54 cultures from 12 cases), simply added small amounts of blood to tubes of Nicolle-Novy-MacNeal medium ("N.N.N."). Row and Knowles (34 cultures from one case) added $\frac{1}{4}$ to 2 c.c. of blood to 20 c.c. of "citrated saline" and then distributed the sediment into N.N.N. medium after incubating the tubes for 24 hours at 22° C.

The low percentage of successful culture by Knowles shows that neither method can be relied upon to give constant results. We have confirmed the findings of Cornwall and LaFrenais[2] that the blood of man is unfavorable to the growth of *Leishmania* and have found that this is true not only for whole blood but for washed red cells and for serum whether fresh or heated for $\frac{1}{2}$ hour at 56° C. There was only an occasional feeble growth with blood and red cells, none with serum alone.

In order to free the blood from red cells and serum the following method was used: 10 c.c. of blood was drawn from a vein at the elbow into one or two cubic centimeters of citrated Locke's solution and immediately expelled into a flask containing 50 c.c. of the same fluid. This diluted blood was centrifuged at a low speed to throw down the red cells only. The supernatant fluid was transferred to another sterile 50 c.c. tube and centrifuged at a high speed. The sediment from this was distributed into tubes of buffered N.N.N. medium adjusted to varying hydrogen-ion concentrations and incubated at 22° C.

*Culture Medium.*—This was the Nicolle-Novy-MacNeal medium[3, 4] with the addition of 0.2 per cent. dipotassium phosphate ($K_2HPO_4$) as a buffer. The salt-phosphate agar was adjusted to hydrogen-ion concentrations varying from $P_H$ 6.8 to $P_H$ 8.2. Defibrinated rabbit blood was added in the proportion of one part of blood to three of agar. Leishman-Donovan bodies in spleen pulp and peripheral blood develop into flagellates throughout the range tested with little detectable difference. Cultures from

[1] Knowles, R., *Indian Jour. Med. Res.*, 1920, viii, 140.

[2] Cornwall and LaFrenais, *loc. cit.*, p. 299.

[3] Novy and MacNeal, *Jour. Inf. Diseases*, 1904, i, 1.

[4] Nicolle and Comte, *Bull. de la Société de Path. Exotique*, 1908, i, 299.

spleen and blood have not yet been attempted at higher and lower concentrations than those indicated. Flagellates grow on medium at least as alkaline as $P_H$ 9.0. In all of these cultures post-flagellate forms may be found including definite Leishman-Donovan bodies. Other forms suggesting Cornwall's second type of "thick tails"[1] are occasionally met with. Cultures on buffered medium remain viable for a considerable time. A successful subculture has been made after 56 days although no flagellates were found in a drop of the fluid used for inoculation. Growth took place on the surface of the medium above the water of condensation, to a height of four centimeters in one instance. The surface of the medium was slightly dulled. Such surface growth seemed especially rich in rosettes and active flagellates.

The results from peripheral blood thus far cultured are as follows: Ten samples of blood have been taken from five different patients and distributed into forty tubes of buffered N.N.N. medium of various hydrogen-ion concentrations and incubated at 22° C. Twenty-nine of these cultures were positive (72.5 per cent.). One or more tubes from nine of the ten samples showed flagellates (90 per cent.). At least one culture was positive from each patient. One case gave positive results after having received 20 c.c. of 0.2 per cent. colloidal antimony sulphide eight hours before the blood was taken. Before the culture she had received in all 121 c.c. of the suspension, equivalent to 0.173 gram of metallic antimony injected intravenously over a period of fourteen days. Another patient had received intravenously 377 c.c. of a similar suspension, equivalent to 0.539 gram of metallic antimony, over a period of thirty-three days. The last dose of 30 c.c. was given three days before the culture was taken.

## SUMMARY.

1. Human red cells and serum are unfavorable to the growth of *Leishmania donovani*.

2. A method is given for removing the red cells and serum from blood before planting.

3. By this method blood cultures have been obtained from nine out of ten samples of blood from five patients, some of them after considerable antimony treatment.

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[1] Cornwall and LaFrenais, *loc. cit.*, p. 299.

4. A modified "N.N.N." medium is suggested.

5. On this medium the Leishman-Donovan bodies from spleen punctures or peripheral blood develop into flagellates at all hydrogen-ion concentrations tested, *i.e.*, between $P_H$ 6.8 and $P_H$ 8.2 and the flagellates grow at least to $P_H$ 9.0.

6. Cultures on this modified medium show post-flagellate forms and perhaps Cornwall's "thick tails."

## ABSTRACTS OF THE COMMUNICATIONS, PACIFIC COAST BRANCH.

*San Francisco, California, February 15, 1922.*

### 136 (1883)

### Further observations on anaphylactoid phenomena from different agents, including histamin.[1]

By **PAUL J. HANZLIK** and **HOWARD T. KARSNER.**

[*From the Department of Pharmacology, Leland Stanford Junior University, San Francisco, Cal., and the Department of Pathology, Western Reserve University, Cleveland, Ohio.*]

Of the twenty-five different agents which were injected intravenously into guinea pigs, the following caused anaphylactoid symptoms: Colloidal arsenic, kaolin, blood charcoal, colloidal iron, ten per cent. sodium chloride (?), tragacanth, toxified agar, lung extract, glacial acetic acid, copper sulphate, fuller's earth, sodium oxalate, sodium citrate, tannin, tartar emetic and histamin. Especially noteworthy were the results after injection of histamin, which produced symptoms with the very small dosage of 0.00011 mgm. per gram of animal. All of these agents, except the chloride and citrate, produced thrombi in the pulmonary blood vessels. The appearance of pulmonary thrombi (platelet) after the injection of histamin agrees with the observation of Dale and Laidlaw, who detected the presence of platelet thrombi in the blood of cats in histamin shock. Histamin also causes agglutination of human, avian, guinea pig, dog and cat red blood corpuscles "in vitro," which is consistent with the formation of thrombi and emboli "in vivo."

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[1] This investigation is supported by a grant from the Therapeutic Research Committee of the Council of Pharmacy and Chemistry of the American Medical Association.

The following agents, caramel (50 per cent.), cane sugar, casein, calcium lactate, lutein extract, horse serum, colloidal gold (sensitive), colloidal gold (protected) and colloidal sulphur (saturated), did not cause anaphylactoid symptoms. Pulmonary thrombi were not demonstrable after cane sugar, sodium chloride, casein, calcium lactate, horse serum and colloidal gold (sensitive).

Cane sugar and sodium chloride (hypertonic solutions) have been advocated and used for the prevention and treatment of anaphylactoid symptoms. The rationale of this is not understood, but is alleged to be concerned with alterations in the physical-chemical properties of the colloids of the blood. Accordingly, attempts were made to treat anaphylactoid symptoms produced by kaolin, histamin, acacia, and beef serum by preliminary injections of 50 per cent. cane sugar and of 10 per cent. sodium chloride. However, the results obtained were uniformly negative as to prevention of symptoms and thrombi.

Intraperitoneal injections were made with the following agents, allowing at least one hour after administration for development of symptoms; agar sol-gel, toxified agar, horse serum, beef serum, lung extract, histamin, colloidal gold (sensitive), colloidal sulphur (saturated), Congo red, glacial acetic acid and copper sulphate. With the exception of copper sulphate, the results were uniformly negative. The intraperitoneal injection of copper sulphate in the same dosage as was used intravenously, caused anaphylactoid symptoms and death in 40 minutes. Pulmonary thrombi were definitely present after intraperitoneal injection of histamin only.

### 137 (1884)

## Urinary excretion of salicyl after the administration of salicylate and salicyl esters.

By **P. J. HANZLIK, FLOYD DE EDS** and **ELIZABETH PRESHO.**

[*From the Department of Pharmacology, Leland Stanford Junior University, San Francisco, Cal.*]

Using sodium salicylate as a control, the excretion of methyl salicylate, acetylsalicylic acid and salicylosalicylic acid (diplosal) was compared in each of three human individuals. Small doses of 0.5 gm. to 1 gm. were used. The mean total excretion of sodium salicylate was 80 per cent., which agrees with the results obtained with very large doses (12 gms.) previously reported. On the other hand, the mean total excretion of salicyl after the administration of the salicyl esters was distinctly less, namely, 60 per cent. Special treatment of the urines for detection and estimation of the undecomposed esters gave contradictory results. Unchanged esters appeared to be present to a small extent only in some urines, absent in other urines. Ethereal extractives of the urines after the administration of methyl salicylate possessed a fruity odor, indicating the presence of the unchanged ester. Larger doses of the esters may give more conclusive evidences along this line. Since all urines were collected until excretion of salicyl was completed, the salicyl unaccounted for appears to have been destroyed, and this is confirmative of previous results with large doses of sodium salicylate. The mean duration of excretion of sodium salicylate and the esters was practically the same, namely, about 48 hours; only the methyl salicylate showing a tendency to somewhat more prolonged excretion (55 hours).

### 138 (1885)

## The effect of the administration of salvarsan in combination with various colloid substances on its toxicity.

By **JEAN OLIVER** and **SO SABRO YAMADA**

[*From the Department of Pathology of the School of Medicine, Leland Stanford Junior University, San Francisco, Cal.*]

In some unpublished articles it has been shown that the ill effects following salvarsan administration may be divided into two types; first, an immediate reaction from which the animal dies suddenly in a convulsive seizure as a result of embolism of its agglutinated red cells, and second, a late death occurring in from two days to as many weeks, which is the result of degenerative lesions in the kidneys and liver. As the former has been shown to be the effect of the physical properties of the salvarsan solution, while the later is the result of its chemical constitution, we have suggested the terms "physical" and "chemical" toxicity for these two types of ill effects. We have also studied the protective action of various colloids on the process of agglutination of red cells in vitro and found that there is a marked inhibition of the agglutination from salvarsan by certain of these substances.[1]

Recently the effect of the administration of salvarsan in mixture with these protective colloids has been studied. An almost complete removal of the physical toxicity of salvarsan has been found. Under our standard conditions an animal rarely survives the injection of more than .27 gram per kilo of 2 per cent. disodium salvarsan. Yet we have repeatedly given doses of .40 gram per kilo of a similar preparation mixed in a 3 per cent. solution of gelatin with no immediate ill effects. Such animals die from the late chemical toxicity of this tremendous dose in the course of a few days.

The late chemical toxicity of salvarsan is also lessened. Under the conditions of our experiments the maximum tolerated dose of salvarsan was found to be .09 gram per kilo, the majority of animals receiving such a dose surviving two weeks. When administered in 3 per cent. gelatin solution, the maximum tolerated dose was found to be .14 gram per kilo. Administration in acacia solution and in serum was found much less effective in reducing the toxicity, the maximum tolerated dose in the former case being .10 gram per kilo.

Repeated injections of large doses are also better tolerated if the salvarsan is administered in gelatin solution. In some previous experiments animals were given .10 gram per kilo every three days. All the animals died, the majority after the second injection, and on microscopic examination showed marked necrosis of their kidneys. Three rabbits receiving the same dose in gelatin solution withstood 4, 6 and 7 doses and lived 9, 16 and 24 days respectively. The kidneys of these animals showed in the first case a slight parenchymatous degeneration with some necrosis, while in the other two no definite lesions were found.

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[1] These articles will appear in an early number of the *Journal of Pharmacology and Experimental Therapeutics.*

139 (1886)

## The so-called permanent polyuria of experimental diabetes insipidus.

By **E. B. TOWNE.**

[*From the Laboratory of Surgical Pathology, Stanford University Medical School, San Francisco, Cal.*]

Several types of operative damage to the pituitary body or to the tuber cinereum of the dog cause a polyuria which lasts about three days. A few observations of more enduring polyuria have been reported. In Crowe, Cushing and Homans's[1] Obs. 34 (partial removal of the anterior lobe and separation of the stalk) the polyuria was still present when the animal was killed six months after operation. Camus and Roussy's[2] dog "Moustachu" was putting out large amounts of urine when killed in the seventh month. Autopsy showed that the tuber cinereum had been punctured and that the stalk had been divided. Matthews[3] twice produced polyuria by introducing through the sphenoid bone a piece of gutta-percha tissue which impinged on the posterior lobe, stalk and tuber cinereum. One experiment lasted nine weeks before the animal was killed. Camus and Roussy[4] reported two dogs with "permanent diabetes insipidus" which were still alive ten and twelve months after the production of a lesion intended to involve only the tuber cinereum. Bailey and Bremer,[5] who produced small lesions of the tuber cinereum, said that in three dogs "the polyuria was permanent." The experiments lasted ten days, six weeks and four months respectively; in the third animal the posterior lobe had been detached from the infundibulum.

The matter at issue is whether the polyuria is due to suppression of the secretion of the pars intermedia, or to injury of a hypothetical nerve center in the tuber cinereum, or to some other cause. The average length of life in the eight observations summarized above was five months. It has been assumed in some of these experiments that the polyuria was permanent. I have made observations which bear on the question of permanency.

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[1] Crowe, Cushing and Homans, *Johns Hopkins Hosp. Bull.*, 1910, xxi, 127.

[2] Camus and Roussy, *Presse méd.*, 1914, xxii, 517.

[3] Matthews, *Arch. Int. Med.*, 1915, xv, 451.

[4] Camus and Roussy, *Comptes Rendus de la Soc. de Biol.*, 1920, lxxxiii, 764; ibid., 901; ibid., 1578.

[5] Bailey and Bremer, *Arch. Int. Med.*, 1921. xxviii, 773.

Dog 4; female, weight 6,135 gm., about 6 months old, had an average daily output of 140 c.c. of urine. Jan. 5, 1920, under ether anesthesia, the pituitary gland was exposed by Paulesco's technic, and an incision was made through the tuber cinereum which opened the third ventricle and completely separated the stalk and gland from the base of the brain. The output of urine rose for three days to about 600 c.c., dropped to 200 c.c. for the following four days, and rose to 1,000 c.c. on the ninth day. From that time until Apr. 1, the output was always above 1,000 c.c. except when it was reduced for a day by injection of pituitary extract. The largest amount was 1,450 c.c. on Feb. 26. From Apr. 1 to May 22 the output varied between 800 and 500 c.c., always tending downward. On June 5 it reached 250 c.c. and remained there until June 24, when the animal was killed. The polyuria had lasted about 20 of the 24 weeks after operation, and during that period her weight rose to 1,250 gm. She was always active and well, but did not come in heat.

At autopsy there was a marked increase in adipose tissue. The uterus and ovaries were infantile. The thyroid and adrenals were microscopically normal. The ovaries showed ova in every stage of development. A block from the floor of the third ventricle, including the tuber cinereum and the attached pituitary gland, was cut in serial sections. The scar of the operative incision was at the lower margin of the optic chiasm anteriorly and at a slightly lower level posteriorly. To this extent the floor of the third ventricle had been reformed by connective tissue without ependymal lining. All of that portion of the pars intermedia which covers the tuber cinereum and the neighboring base of the brain (Tilney's pars tuberalis) had been detached with the stalk. The pars anterior appeared normal, with about the usual proportion of chromophile and chromophobe cells. The pars nervosa was atrophic and invaded by cells of the pars intermedia type. Surrounding the altered pars nervosa was an empty space which, from the evidence of a few sections, had been filled with colloid material. Outside this space was a zone of pars intermedia cells. Posteriorly these cells were separated from the base of the brain by scar tissue, but anteriorly, at the point where the connective tissue portion of the floor of the ventricle joined the normal floor, there was no intervening scar tissue. Here a mass of pars intermedia cells, continuous anteriorly with pars anterior cells, had invaded the base of the brain so that the cells lay immediately under the ependymal lining. The majority of these cells were chromophobe and arranged in alveoli containing colloid, but among them were occasional eosinophiles similar to those in the adjacent anterior lobe.

In other experiments I have produced polyurias that ended in the third, fourth or fifth month, but sections have shown that the stalk division had not been made sufficiently high to detach all the pars tuberalis cells from the base of the brain, and that this epithelium had proliferated above the scar. Only in Dog 4 were all of these cells detached with the gland. The sections of this animal are interpreted as meaning that the anterior and superior portion of the pars intermedia reunited with the uninjured floor of the third ventricle without any intervening scar tissue, so that there was nothing to prevent pars intermedia products from passing into the ventricle, if that be the method of secretion.

I am not ready to interpret these findings as they effect the question of the cause of experimental polyuria. They indicate that a stalk division high enough to detach all epithelium from the base of the brain and completely destroy the tuber cinereum does not necessarily result in a permanent polyuria, and that the true permanency of previously reported results of such experiments is open to question.

# SCIENTIFIC PROCEEDINGS.

## Abstracts of Communications.

### One hundred twenty-third meeting.

*The College of the City of New York, April 19, 1922.*
*President Wallace in the chair.*

140 (1887)

### Basal metabolism in relation to body surface at different ages with special reference to prematurity.

By **FRITZ B. TALBOT** and **WARREN R. SISSON.**

[*From the Massachusetts General Hospital, Boston, Mass.*]

In 1902 Rubner[1] brought forth evidence that the heat loss of the body was proportional to its surface area. This became known as Rubner's law of surface area. The principle was not new as half a century earlier Regnault and Reiset noted that the heat production of sparrows per unit of weight was ten times greater than that of fowls, and concluded that it was due to the fact that the smaller animals had a relatively larger body surface, and, consequently, lost more heat than larger animals, with relatively less surface. In 1913 Rubner[2] presented further evidence to show that the even heat production per unit of body surface was not dependent on the active tissues within the organism.

In 1908 Schlossmann and Murschhauser[3] in their investigations, in which the effect of muscular exercise on the metabolism was eliminated, found that instead of the 1,000 calories per square meter of body surface, absolutely quiet healthy infants produced only 866 calories in 24 hours.

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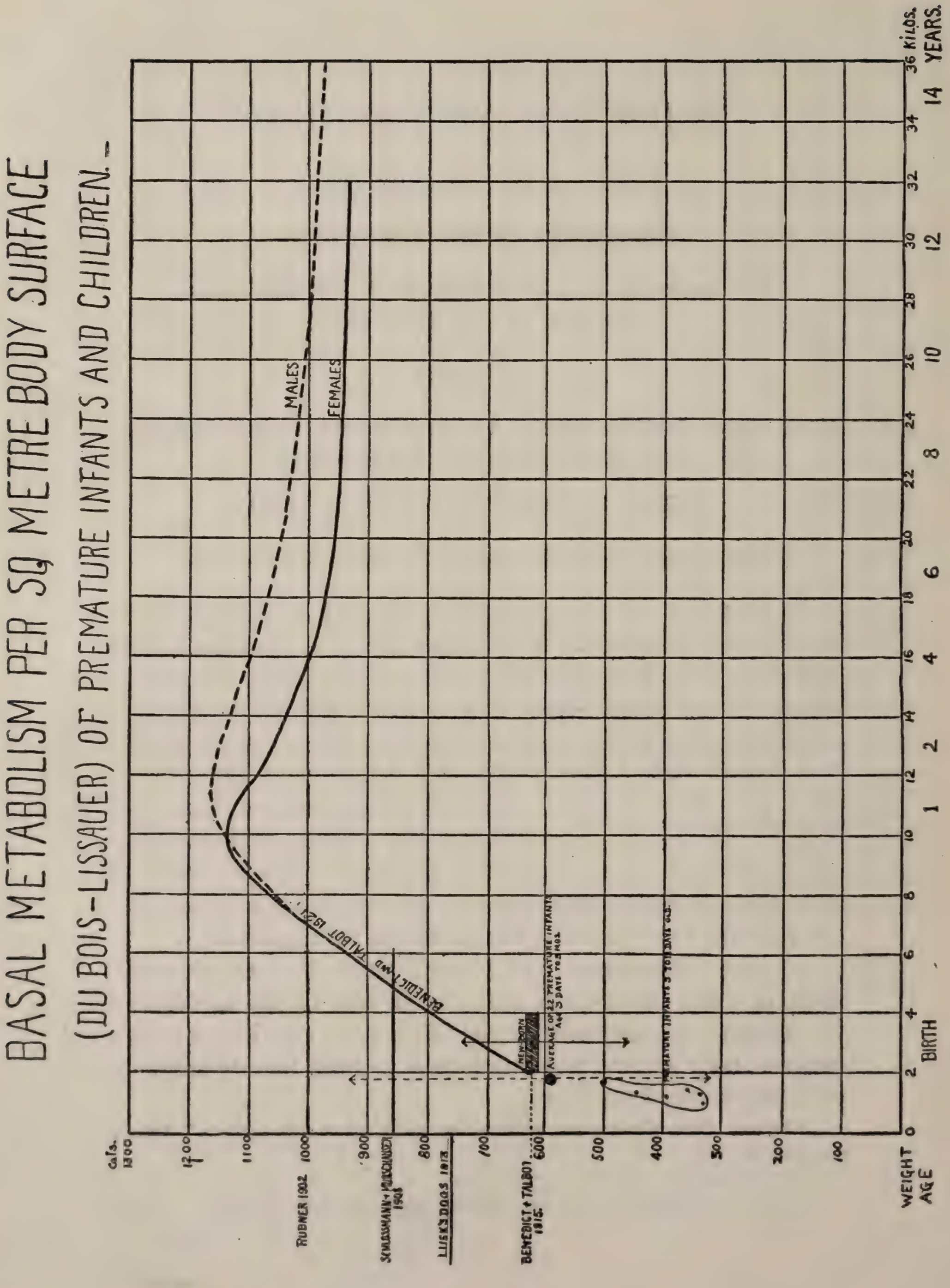
[1] Rubner, "Die Gesetze des Energieverbrauchs bei der Ernahrung," Leipzig and Vienna, 1902.

[2] Rubner, *Arch. f. Physiol.*, 1913, p. 240.

[3] Schlossmann and Murschhauser, *Biochem. Zeitschr.*, 1908, xiv, 385.



BASAL METABOLISM PER SQ METRE BODY SURFACE (DU BOIS-LISSAUER) OF PREMATURE INFANTS AND CHILDREN.

Lusk in 1913[4] showed that sleeping dogs could produce as low as 750 calories, while Benedict and Talbot in 1915[5] found the average heat production per unit of surface of 105 new-born infants was 612 calories.

In 1921 Benedict and Talbot[6] constructed a curve of the metabolism from birth to puberty from studies based on a new series of 256 infants and children, see following chart. This shows a rapid increase from 612 calories in new-born infants to 1,170 calories in boys of about one year of age, weighing 11 kilograms.

During the past year we have studied, with the assistance of Miss Margaret Moriarty and Mrs. A. J. Dalrymple, the heat production of 22 premature infants. Since premature infants have proportionally more body surface per unit of weight than larger subjects, they should produce more heat per unit of surface. On the contrary, our studies show quite the reverse. The average heat production per unit of surface of the 22 premature infants studied was 597.3 calories in 24 hours. We have included in this group premature infants of 3 days to 3 months of age. A critical analysis will be made of these cases later, but it seems probable that the few older infants of this series will fall in the category of malnutrition, in which the metabolism is usually high.

Six of these 22 infants were studied during the first 11 days of life and in these instances the metabolism was extraordinarily low. They are represented in the lower part of the chart by dots inside the light line.

This data is presented as further evidence that Rubner's law of the constant relationship between heat production and body surface is not a physiological law, and that it does not hold true in infants.

### 141 (1888)

## Studies on salt action. IV. The mutual influence of acidity and salt concentration upon bacteria.

By **C.-E. A. WINSLOW** and **I. S. FALK.**

[*From the Department of Public Health, Yale School of Medicine, New Haven, Conn.*]

Four years ago[1] we presented certain preliminary results of studies on the toxic influence of calcium and sodium salts upon *Bact. communis*; and two years ago one of us[2] called attention to the possible influence upon such salt effects of the reaction of the solution.

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[4] Lusk, *Jour. Biol. Chem.*, 1913, xiii, 450.

[5] Benedict and Talbot, Carnegie Institution of Washington Publication No. 233.

[6] Benedict and Talbot, Carnegie Institution of Washington Publication No. 302.

[1] Winslow and Falk, PROC. SOC. EXP. BIOL., 1918, xv, 67.

In our earlier experiments the reaction of the distilled water and salt solutions to which the bacteria were exposed was not controlled though the media were in general alkaline ($P_H$ 8.0–9.0). Under these conditions we obtained the following results with various concentrations of NaCl and $CaCl_2$.

PER CENT. OF BACTERIA SURVIVING AFTER 9 HOURS.

| | Tonicity of Salt. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0. | 0.1 | 0.2 | 0.5 | 1.0 | 3.0 | 5.0 | 7.0 | 10.0 |
| NaCl.......... | 89 | 115 | | | 83 | 64 | 58 | 20 | < 1 |
| $CaCl_2$.......... | 89 | 70 | 92 | 45 | 22 | < 1 | < 1 | < 1 | < 1 |

Later experiments, in which the hydrogen-ion content was maintained at various desired levels by repeated readjustment, gave quite different results, as indicated below.

PER CENT. OF BACTERIA SURVIVING AFTER 9 HOURS.

| | PH. | | | | | |
|---|---|---|---|---|---|---|
| | 5.0 | 5.5 | 6.0 | 7.0 | 7.5 | 8.0 |
| Water.............................. | 82 | | 106 | 54 | | 12 |
| 5.0 isotonic NaCl.................... | 27 | 20 | 87 | 76 | 8 | 9 |
| 1.0 isotonic $CaCl_2$.................... | 134 | | 128 | 106 | | |

These results (which are all based on averages of a sufficient number of tests to eliminate chance variations) were at first highly puzzling. In the unadjusted alkaline solution 5.0 isotonic NaCl was moderately toxic and 1.0 isotonic $CaCl_2$ highly toxic. At adjusted $P_H$ values on the other hand 5.0 isotonic NaCl remained at practically all reactions slightly more toxic than water but 1.0 isotonic $CaCl_2$ entirely lost its toxicity and became actually stimulating, at least at $P_H$ values between 5.0 and 7.0. It appeared therefore that neither 5.0 isotonic NaCl nor 1.0 isotonic $CaCl_2$ is in itself toxic at adjusted $P_H$ values between 6.0 and 7.0; and that at an adjusted $P_H$ much over 7.0 both salts, as well as distilled

[2] Falk, PROC. SOC. EXP. BIOL., 1920, xvii, 210.

water, are definitely toxic. What conditions could have existed in the earlier work, at uncontrolled $P_H$, to make 1.0 isotonic $CaCl_2$ toxic and 5.0 isotonic NaCl nearly non-toxic?

The key to this riddle, first suggested by the experiments reported here two years ago, has been found in the influence of salt content upon the power of a bacterial suspension to regulate its reaction. We find, as shown below, that in an unadjusted alkaline water solution containing bacteria the reaction quickly reverts toward the neutral point, while in a similar isotonic $CaCl_2$ solution the reversion is much slower. In slightly acid solutions ($P_H$ 6.0) this difference in behavior is not apparent.

| | Distilled Water. | | | Isotonic $CaCl_2$ | | |
|---|---|---|---|---|---|---|
| | $P_H$ Initial. | 9 Hrs. | Per Cent. Bacteria Surviving After 9 Hrs. | $P_H$ Initial. | 9 Hrs. | Per Cent. Bacteria Surviving After 9 Hrs. |
| Unadjusted. | 9.2 | 8.0 | 91 | 9.2 | 8.9 | $< 1$ |
| Adjusted... | 6.0 | 6.1 | 76 | 6.0 | 6.5 | 90 |

These last experiments indicate again that at a $P_H$ controlled between 6.0 and 7.0 neither water nor 5.0 isotonic $CaCl_2$ has toxic action; that in water with an initial $P_H$ over 9.0 the reaction falls to about 8.0 in 9 hours and no toxic effect is manifest; and finally that in an isotonic $CaCl_2$ solution of initial $P_H$ over 9.0 the reaction changes but slightly and the bacteria show the high mortality to be expected in such an alkaline solution. The only anomaly that remains is the fact that the alkalinity of the unadjusted water solution although it falls considerably still remains rather high. In these last experiments a $P_H$ of 8.0 after 9 hours is non-toxic while as previously indicated a $P_H$ maintained at 8.0 throughout an experiment is toxic. We may explain this on the probable assumption that in a solution whose reaction is being brought toward neutral by the regulating action of contained bacteria there are zones immediately surrounding the bacterial cells which have an alkalinity lower than the average for the suspension as a whole. Thus our $P_H$ readings in such a suspension do not represent the actual concentration of hydrogen ions about the cell as they do in a suspension of artificially controlled reaction.

It appears then that the toxic effect of $CaCl_2$ reported in our first experiments is an indirect one and is exerted only in an alkaline solution in which it interferes with the regulative action exerted by bacterial cells upon the reaction of a water or NaCl solution.

### 142 (1889)

## Studies on salt action. V. The influence of various salts upon bacterial growth.

By C.-E. A. WINSLOW and MARGARET HOTCHKISS.

[*From the Department of Public Health, Yale School of Medicine, New Haven, Conn.*]

Previous studies on salt action conducted in this laboratory have dealt with the effect of certain mineral salts upon the death rate of bacteria in water suspension.[1] The present investigation relates to the influence of various salts upon growth in a one per cent. pepton solution. The pepton used contained about 4 per cent. ash and the solution had a reaction of $P_H$ 6.8–7.0. The salts studied were added in the form of chlorides in varying concentration. The solutions were inoculated with *Bact. communis* and incubated at 37° C., the rate of growth being determined by comparing the turbidity produced with standard suspensions of dead bacterial cells. Check determinations by the plate method indicated the substantial accuracy of this procedure.

Twenty-three salts in all were studied and the limiting toxicity determined as indicated below.

The results in general confirm those reported by Matthews[2] for *Fundulus*, and Eisenberg[3] for bacteria; and it is evident, as the former author pointed out, that there is a rough general relationship between toxicity and solution tension. We are making a further analysis of the relation between the toxic action of these salts and their other physico-chemical properties.

The new point brought out in our studies is the general occurrence of a definitely stimulating action, exerted by concentrations of salts below the inhibitive level. In the case of 15 out of the

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[1] Winslow and Falk, PROC. SOC. EXP. BIOL., 1922, xix, 311.

[2] *Am. Jour. Physiol.*, 1904, x, 290.

[3] *Centr. f. Bakt.*, Abth. I, 1918, lxxxii, 69.

SALT CONCENTRATIONS WHICH LIMIT BACTERIAL GROWTH (INCUBATION PERIOD—THREE DAYS).

| Salt. | Molar Conc. No Growth. | Growth. | Salt. | Molar Conc. No Growth. | Growth. |
|---|---|---|---|---|---|
| $HgCl_2$ | .00001 | .000005 | $TlCl$ | .005 | .001 |
| $CdCl_2$ | .0001 | .00005 | $NiCl_2$ | | |
| $CeCl_2$ | .0001 | .00005 | $SnCl_4$ | | |
| $AlCl_3$ | .0005 | .0001 | $TiCl_3$ | .01 | .005 |
| $PbCl_2$ | | | | | |
| $CoCl_2$ | | | $MnCl_2$ | .05 | .025 |
| | | | $BaCl_2$ | .25 | .1 |
| $FeCl_3$ | .001 | .0005 | $CaCl_2$ | .5 | .25 |
| $FeCl_2$ | | | $MgCl_2$ | .5 | .25 |
| $CuCl_2$ | | | $SrCl_2$ | 1.0 | .25 |
| $ZnCl_2$ | | | | | |
| | | | $LiCl$ | 1.0 | .5 |
| | | | $NH_4Cl$ | 1.0 | .75 |
| | | | $NaCl$ | 2.0 | 1.0 |
| | | | $KCl$ | 2.0 | 1.0 |

23 salts studied we found a concentration which caused more rapid growth than occurred in the plain pepton solution, the stimulating salts including not only K, Na, $NH_3$, Li, Sr, Mg, Ca and Ba, but such toxic salts as those of Ti, Sn, Ni, Pb, Ce and Hg. The stimulating concentrations with the latter salts were of course exceedingly low (.00005 molar in the case of Pb, .00001 molar in the case of Ce, .000005 molar in the case of Hg) while with K and Na .25 molar concentrations were stimulating. It is very possible that stimulating concentrations of the other eight salts could have been established by more exhaustive study.

### 143 (1890)

## A ten-year-old strain of fibroblasts.

By **ALBERT H. EBELING** (by invitation).

[*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*]

A strain of fibroblasts, obtained from the heart of a chick embryo on January 17, 1912, has completed the tenth year of its life *in vitro*. On April 19, 1922, our incubators contained about 60 cultures which represented the 1906th generation of the connective tissue cells. Their growth is as rapid as during the past years, each fragment generally doubling its volume in 48 hours. In 10 years, more than 30,000 cultures have been derived from a fragment of heart less than 1 cubic millimeter in size. This demonstrates first, that the cells transform the food stuffs in their medium into protoplasm. Second, under the conditions of the experiments, the cells are no longer subject to the influence of time, as they are when living within the organism, and demonstrate that they are potentially immortal. The cells have now exceeded the average life of chickens, which disposes of criticisms on this point.

Pure cultures of cells are important in studying biological problems. The strain responds readily to changes in the composition of the culture medium by modifying its rate of proliferation. By perfecting the technique, it could be used as a reagent for detecting substances contained in the humors which have the power of activating or decreasing the rate of cell proliferation; and further, to investigate the interactions of the cells and their medium, which are still incompletely known.

### 144 (1891)

## Bio-radiological studies.

By **HERBERT RUCKES** and **ARTHUR W. FUCHS** (by invitation).

[*From the Department of Biology, College of the City of New York.*]

Animals in which arteries, veins and ducts of various organs are injected with salts of heavy metals, such as mercury, barium, lead, gold and silver are skiagraphed and subsequently studied in either plane view or by means of stereoscopic rœntgenograms. The method is especially valuable for the study of animals from the view point of comparative anatomy, Such preparations show clearly first, the general construction of the arterial or venous system and secondly the minute ramifications of the respective arteriole systems on and within the organs. Probably the most valuable use that this method has is in the study of developing systems and ducts in embryos. Here the homologies of the embryonic blood vessels and the adult arteries and veins can readily be demonstrated.

Barium sulfate and litharge are the best injection media. Baruim sulfate is prepared by precipitating it from barium hydroxide with ammonium sulfate, the precipitation being done at about 57° C. This gives a very fine flocculent mass that will enter many of the smaller arteries and veins. An aqueous suspension is used. Red lead and litharge are employed in either aqueous suspension or in oil.

The plates are exposed to the x-rays varying lengths of time depending on the size and density of the object and the amount of current used.

The paper is illustrated with lantern slides of the prepared objects.

## 145 (1892)

## The selective bacteriostatic activity of sulfanilic acid.

By **JOHN W. CHURCHMAN.**

[*From the Department of Hygiene, Cornell University Medical School, New York City.*]

In a recent communication to this Society report was made of a selective bacteriostatic property exhibited by acid fuchsin. From a study of the effect of this dye on spore-bearing gram-positive aërobes and on the commoner gram-negative bacteria its selective activity was shown to be just the reverse of that exhibited by gentian violet and the other basic tri-phenyl-methane dyes: the latter kill the gram-positive aërobes and spare gram-negative bacteria, while acid fuchsin kills the gram-negatives and spares the gram-positive aërobes.

Magenta is one of the basic tri-phenyl-methanes and has the same effect on bacteria as gentian violet. From magenta, acid fuchsin differs only (so far as chemical structure is concerned) by the presence in its molecule of sulphonic-acid groups. Since the two dyes are—with this exception—identical, and since they have—so far as gram-positive aërobes and the commoner gram-negative bacteria are concerned—exactly opposite effects, it was clearly indicated to determine whether the sulfonic acid groups in acid fuchsin were responsible for its ability to kill gram-negative organisms while sparing gram-positive aërobes.

The behavior of sulfonic acid groups was tested by a study of sulfanilic acid. It was found that this substance behaves exactly like acid fuchsin: the commoner gram-negative bacteria, when exposed to it at 45° C. are readily killed, gram-positive aërobes under the same treatment remaining uninjured. This would seem to be proof that the reverse selective property of acid fuchsin (reverse, that is to say, as compared with gentian violet) depends on the sulfonic-acid groups which it contains.

Since opposite selective activities have thus been demonstrated for two dyes (magenta and acid fuchsin)—one of which kills gram-positive spore bearers and spares gram-negatives, while the other does just the reverse—and since the chemical group in the latter substance which is responsible for its selective activity has been determined, data would seem to be at hand for the determination of the fundamental cause of the difference between these two types of organisms.

### 146 (1893)

## Detoxication in the organism of the fowl.

By CARL P. SHERWIN and JAMES H. CROWDLE.

[*From the Department of Chemistry, Fordham University, New York City.*]

Various organic compounds more or less toxic to the organism have been fed to human beings as well as to many of the lower animals, but little work of this kind has been carried out with the fowl.

Previous investigation has shown that the first action of the animal body is an attempt at complete oxidation of the foreign molecule. Should this fail, an effort is next made to render the compound less toxic by means of reduction with or without subsequent oxidation. If neither of these types of reactions sufficiently alters the foreign substance, recourse is finally had to a synthetic type of reaction by which the original compound is joined in most cases with another compound or radical.

It may be said in general that the effect of oxidation or reduction upon a compound of this kind is to produce either an alcohol or an acid. The acids then are usually detoxicated by being joined with glycocoll, glutamine, ornithine or glycuronic acid, while the alcohols are combined with either the sulphate radical, glycuronic acid or cysteine. Besides this we have methylation of pyridine compounds, acetylation of amino compounds, and the combination of amino compounds to form uramino acids.

For our work we chose compounds which illustrate type reactions only and which in some instances undergo entirely different processes of detoxication in different organisms.

1. Phenylacetic acid was fed to check up some of the older work, and it was found that in the organism of the dog phenylactic acid is combined with glycocoll and appears in the urine as phenaceturic acid. In the human organism, however, it is joined with glutamine and is excreted as phenylacetyl-glutamine, and when fed to chickens it is excreted as phenacetornithuric acid, *i.e.*, in combination with ornithine.

2. Benzaldehyde, both when fed to human beings as well as to lower animals is oxidized to benzoic acid and excreted as hippuric acid.

We fed a total of 3 gms. to a hen and isolated from the excreta 0.5 gm. of ornithuric acid (2 molecules of benzoic acid combined with 1 molecule of ornithine.)

3. Para-hydroxy benzaldehyde when fed to human beings is oxidized to para-hydroxy benzoic acid and is excreted as such, but when fed to lower animals it is as a rule detoxicated further by being combined with glycocoll and is excreted at least in part as para-hydroxy hippuric acid. The chicken, however, employs the same means of detoxicating this compound as does the human being.

After feeding 3.6 gms. of para-hydroxy benzaldehyde to a hen we isolated 1.5 gms. para-hydroxy benzoic acid from the excreta but no conjugation product appeared.

4. Phenylpropionic acid, when fed to animals, undergoes beta oxidation and is converted into benzoic acid. After feeding 2.5 gms. of phenylpropionic acid to a hen we extracted from the excreta about 0.5 gm. benzoyl ornithine together with some free benzoic, showing that beta oxidation takes place as readily in the organism of the fowl as in the bodies of other animals.

5. It is generally believed that cinnamic acid by a preliminary process of reduction is first converted by the animal body into phenyl-propionic acid, then by subsequent oxidation into benzoic acid. This type of reaction is apparently employed by the fowl for the detoxication of cinnamic acid, for we isolated somewhat more than 0.5 gm. benzoyl ornithine from the excreta after feeding a hen 3 gms. of cinnamic acid.

6. Nitro benzene when fed to animals is extremely toxic, but is detoxicated to some extent by a simultaneous oxidation and reduction through which it is converted into para-amino phenol. We fed 0.5 gm. nitro benzol to a hen. The chicken died at the end of 12 to 14 hours, but qualitative tests were obtained for para amino phenol in the excreta.

Meta-amino benzoic acid undergoes two different kinds of reactions in the animal body. When fed to dogs it is detoxicated to some extent by being joined with glycocoll to form meta-amino hippuric acid. When fed to rabbits, however, it is largely detoxicated by undergoing combination with urea to form a meta-uramino benzoic acid. When fed to fowls the acid seems to undergo a process of acetylation whereby acetic acid is joined onto the amino group of the acid with the splitting out of water resulting in the formation of meta-acetyl-amino benzoic acid.

We fed 4.5 gms. meta-amino benzoic acid to a chicken and recovered 1.8 gms. meta-acetyl-amino benzoic acid.

### 147 (1894)

## Merogony experiments on sea-urchin eggs.

By **ROBERT CHAMBERS** and **HIROSHE OHSHIMA**.

[*From the Marine Biological Laboratory, Woods Hole, Mass.*]

By merogony in the broader sense is meant the fertilization and development of egg fragments whether nucleated or not.

By means of the more accurate method of using a mechanical apparatus for microdissection an attempt was made to repeat the work of earlier investigators (O. and R. Hertwig, Boveri, Driesch, Morgan, Loeb, Wilson and others) especially for the purpose of cross-fertilizing egg fragments of the sea-urchin and sand dollar.

Owing probably to the lateness of the season the cross-fertilization experiments were unsuccessful.

However, the following results were obtained in the self-fertilization of sea-urchin egg fragments which indicate that the size of the nucleus in the swimming larvæ depends directly upon the initial size of the nucleus in the fertilized egg fragment whereas the size of the larva bears no direct relation either to the size of the nucleus or to the initial amount of cytoplasm in the fertilized egg. Mature eggs were deprived of their nuclei by cutting them out together with a minimum amount of cytoplasm. The non-nucleated fragments were about 4/5 the size of the entire eggs. These, when fertilized, developed into dwarf larvæ of about half the size of the control and with abnormally small nuclei. Other eggs were deprived of more than half of their cytoplasm. These, upon fertilization, developed into dwarf larvæ of about half the size of the control but possessed nuclei equal in size to that of the control.

### 148 (1895)

## Halophilic bacteria.

By **WILLIAM W. BROWNE.**

[*From the Department of Biology, The College of the City of New York.*]

The salt-fish industry of the United States suffers a large annual loss as a result of the salt fish developing a red coloration during the summer or when stored under warm moist conditions. Work undertaken by the U. S. Bureau of Fisheries has demonstrated that the red coloration is due to the growth of two microorganisms whose origin is the solar evaporated sea salt with which the fish are cured. The coloration may vary from a pale opaque pink to a deep transparent crimson due to the harmonious intergrowth of a spirochete producing a pale pink coloration and a bacterium producing a transparent red coloration. Likewise their separation into pure culture is difficult. These organisms exhibit very decided helio-, thermo-, and halophilic characteristics. The optimum concentration of salt for growth forms is saturation, growing luxuriantly on heavily salted fish, brine, sea salt, and saturated salt-fish agar. No growth appears on media containing less than 16 per cent. sea salt by weight. The shape, size, and motility of both forms is dependent upon the salt concentration of the medium, varying from the largest form found in saturated media (14 micra) to the spherical forms (2 micra) found in media of 18 per cent. concentration with all the intermediate forms between. The motility varies from the actively motile long forms, through a tumbling motion in the intermediates, to the non-motile spheres. The amount, character and coloration of the colonial growth does not seem to be affected by the changes in the concentration. Due to their extreme halophilic requirements, the staining of these organisms is very difficult. The optimum temperature for growth is 50° to 55° C. Both forms are strictly aërobic and produce neither gas nor acid in carbohydrates. Both forms will tolerate indefinite exposure to the brightest sunlight. In all probability the brilliant pigmentation is a protection against the bright sunlight in the salina of the tropics where the sea salt is produced. Likewise their tolerance to heat and salt indicates such an origin. Influenced by low temperatures and accumulation of metabolic products these organisms suffer a temporary loss of pigment which is regained on reincubation at high temperatures. In the spirochete this loss of pigmentation is accompanied by the formation of coccoid bodies. The peculiar characteristics of these organisms augmented by bacteriological examinations of the sea salt and observations at the point of production have demonstrated that reddening of salted fish is due to the *Spirocheta halophilica* and the *Bacterium halophilica* which are present in the solar evaporated sea salt with which the fish are cured. Sea salts from all over the world contain similar organisms.

### 149 (1896)

## An appliance to be used with a gasometer for recording the volume of each expiration.

By CAMERON V. BAILEY.

[*From the Department of Biochemistry, New York Post Graduate Medical School and Hospital, New York City.*]

The appliance, as demonstrated, is used with a gasometer in which the bell is suspended by a wire passing over a grooved wheel and continuous with a length of steel measuring-tape, which in turn suspends the counterweight.

The appliance consists of a large and a small grooved wheel mounted on a single axle; from the small wheel a silk thread suspends a double magnet fitted with a hinged iron armature, which, on magnetization, firmly grasps the measuring tape of the gasometer. From the large wheel is suspended a counterweight which outbalances the magnet, permitting the weight to sink to the bottom of its guiding channel.

The tube leading to the gasometer carries an expiratory flutter valve; from the subject's side of this valve, a short take-off leads to a large tambour which operates a riding-arm, making and breaking a current during expiration and inspiration respectively.

At the beginning of expiration, this contract is made a fraction of a second before the gasometer bell begins to move; a relay closes the current which operates the magnet, causing it to firmly grasp the steel tape and ride with it during its full excursion. At the beginning of inspiration, the tambour breaks the circuit, the magnet releases the tape, and the counterweight drops to its original position. The counterweight carries a pen which records its movements on a revolving drum.

### 150 (1897)

## Observations on the reaction of the blood of infants with acute intestinal intoxication with the phosphotungstate reagent.

By JEROME L. KOHN.

[*From the Pathological Laboratory, Department of Physiological Chemistry, Mt. Sinai Hospital, New York City.*]

In a previous paper[1] on chemical examinations of the blood in cases of infants and children with acute toxic gastro-intestinal symptoms (cholera infantum), it was noted that in some an intense blue color was obtained using only the phosphotungstic reagent after the uric acid had been precipitated with silver lactate. This blue color was often six to eight times more intense than that obtained with the uric acid alone. The reaction was not noted in normal controls. At that time we stated that we did not know whether phenol was the cause of this reaction.

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[1] Schwarz, H., and Kohn, J. L, *Am. J. Dis. Chil.*, 1921, xxi, 471.

That the so-called phenol reagent (phosphotungstate-phosphomolybdic reagent) first described by Folin and Denis[1] was not specific was shown by Tisdale,[2] Gortner and Holmes[3] and others. They showed that there were other substances which might be present in the blood that gave the same color reaction, hence interfering with the purpose of the original test. A deep blue color is also given by lactic acid, by indol, indol derivatives, protein derivatives and many other substances.

In order to eliminate at least one group of these substances, we determined the amino-acid nitrogen and the peptid nitrogen in some of these cases, using the method described by Van Slyke and Whipple.[4] Although the urea was high in some of these cases the amino-nitrogen and the peptid-nitrogen values were normal, or slightly above normal. One must remember, however, that there may be some toxic protein derivative products or amino acids present which are very toxic in small amounts, although not sufficient to perceptibly increase the amino or peptid nitrogen in the blood.

| Hospital No. | Mgs. per 100 c.c. | | | Remarks. |
|---|---|---|---|---|
| | Amino N. | Peptid N. | Urea N. | |
| 212138 | 12 | 14 | 19.6 | Recovered |
| 212031 | 12 | 14 | 15. | Died |
| 212352 | 14 | 18 | 40. | Died |
| 212338 | 14 | 17 | 19.6 | Died |
| 212410 | 15.4 | 17.1 | 14.2 | Recovered |

### 151 (1898)

## The influence of sodium citrate on peristalsis.

By **WILLIAM SALANT** and **NATHANIEL KLEITMAN.**

[*From the Department of Physiology and Pharmacology, University of Georgia, Augusta, Georgia.*]

The Trendelenburg method was used for the study of intestinal movements in anesthetized animals. Doses of 30–60 mgs. sodium citrate per kilo given intravenously stimulated the contractions of the small as well as of the large intestine, the effect lasting several minutes. Tonus and the rhythmic contractions were increased, but in dogs the effect on tonus predominated. Repetition of dose usually produced greater effects in cats and dogs, but in the rabbit it caused relaxation of the intestine and abolition of the rhythmic movements. This depression was followed, however, by gradual recovery. After intramuscular injections of very large doses of sodium citrate marked and prolonged stimulation occurred; both tonus and rhythmic movements were greatly augmented.

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[1] Folin, O. and Denis, W., *J. Biol. Chem.*, 1912, xii, 239.

[2] Tisdale, F. F., *J. Biol. Chem.*, 1920, xliv, 409.

[3] Gortner, R. A., and Holmes, G. E., *J. Am. Chem. Soc.*, 1920, lxii, 1678.

[4] Whipple, G. H., and Van Slyke, D. D., *J. Exp. Med.*, 1918, xxviii, 213.

The action of citrate was also tested after the division of both vagi. The effect was usually the same as when the salt was given to animals with both vagi intact. Sodium citrate injected after atropine failed to stimulate the intestine, but when pilocarpine was injected after atropine, the subsequent administration of citrate produced temporary inhibition of the intestinal movements and marked decrease of tonus. If more pilocarpine is given and a sufficient interval of time allowed to elapse after the atropine, the usual effect upon the intestine may occur after the injection of citrate.

### 152 (1899)

## The action of sodium citrate on the central nervous system.

By **WILLIAM SALANT** and **NATHANIEL KLEITMAN**.

[*From the Department of Physiology and Pharmacology, University of Georgia, Augusta, Georgia.*]

Maxwell[1] reported experiments in which sodium citrate applied to the cortex of the cerebrum in rabbits was without any effect, but when injected into the white matter underneath produced some of the symptoms of citrate poisoning. These observations were extended later by Robertson and Burnett[2] who made similar studies on the cerebellum. They likewise found that a reaction was obtained only when the solution of sodium citrate was injected into the white matter. The symptoms were with few exceptions the same as those following toxic doses of citrate when given subcutaneously or by mouth. This led them to conclude that they were mainly of cerebellar origin.

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[1] Maxwell, *Jour. Biol. Chem.*, 1906, ii, 183.

[2] Robertson, *Journ. Pharm. Exp. Therap.*, 1911–12, iii, 635.

We made a study of citrate in a series of experiments on cats in which sodium citrate was given after removal of the cerebrum. The subcutaneous injection of toxic doses failed to cause the symptoms usually produced by citrate. Tonic and clonic convulsions, muscular twitching and other symptoms produced by the same doses of citrate in our controls were absent in all of our decerebrated animals.

Tests made on frogs have shown, however, that convulsions and muscular effects may be produced in these animals in the absence of the cerebrum and of the rest of the brain. But when the spinal cord was destroyed no convulsions were observed. Toxic doses of citrate given after division of one sciatic failed to produce spasms of the corresponding leg, and the symptoms produced by citrate disappeared after section of the sciatic.

### 153 (1900)

## Alkaloid actions as test for synapse-function in insects.

By W. J. CROZIER.

[*From the Zoölogical Laboratory, Rutgers College, New Brunswick, N. J.*]

Injection of strychnine solution, even at saturated concentration (0.5 c.c.), in a series of sphingid caterpillars (genera *Samia*, *Automeris*, *Ceratomia*), fails to induce "reversal of inhibition"; and save in the case of those species normally the most excitable it fails to induce any opisthotonic symptoms. Opisthotonic curvature (spasmodic) can be induced, however, by tetraethylammonium chloride. General excitation is produced by a variety of neurophil substances (but not by creatin). Only with atropine is one able to bring about reversal of inhibition in the use of antagonistic muscle groups; it is in this case very clearly shown in the behavior of the prolegs, which no longer react to embrace an object touching the skin between the members of a pair, but instead are pulled widely apart after such stimulation, with their terminal combs retracted.

By the action particularly of pilocarpine, it can be shown that in species normally sluggish, responding mildly to external excitation, the much more violent type of behavior characteristic of species armed with urticant spines may be induced through the effect of neurophil drugs. Therefore the effect of these substances is brought about in relation to nervous pathways already existing. And a suggestion is had as to the basis of behavior differences in species structurally related.

The failure of strychnine to produce its "typical" effects, in these insects, coupled with the observed "reversal" under atropine, points to possible chemical differentiation of the synaptic homologues in insects, and argues for caution in the use of drugs as a test for synapse-function in invertebrates.

### 154 (1901)

## Selective pairing in gammarids.

By **L. H. SNYDER** and **W. J. CROZIER.**

[*From the Zoölogical Laboratory, Rutgers College, New Brunswick, N. J.*]

Studies on the sexual coupling of organisms have shown it necessary to recognize that association of mates may be selective rather than random. It is evident that such selective coupling may have important evolutionary consequences.[1] The problem of selective coupling on the basis of somatic characteristics, however, is an entirely different problem from that of selective union of germ cells. This point must be clearly in mind; the distinction has occasionally lapsed in discussions of the topic.[2]

It has been shown by Pearl and by Jennings that paramœcia assort with respect to size; and the nature and effects of this assorting have been pointed out. More recently it has been shown that the nudibranch *Chromodoris zebra*, which practises internal fecundation, shows a rather high degree of assortative mating according to size.[1] Crozier pointed out some of the likely consequences of large individuals mating with large, and small with small, and he showed that an adaptive result is probably thus attained in a purely mechanical, automatic manner.

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[1] Wright, S., *Genetics*, 1921, vi, 144.

[2] Cf. Jones, D. F., *Biol. Bull.*, 1920, xxxviii, 251.

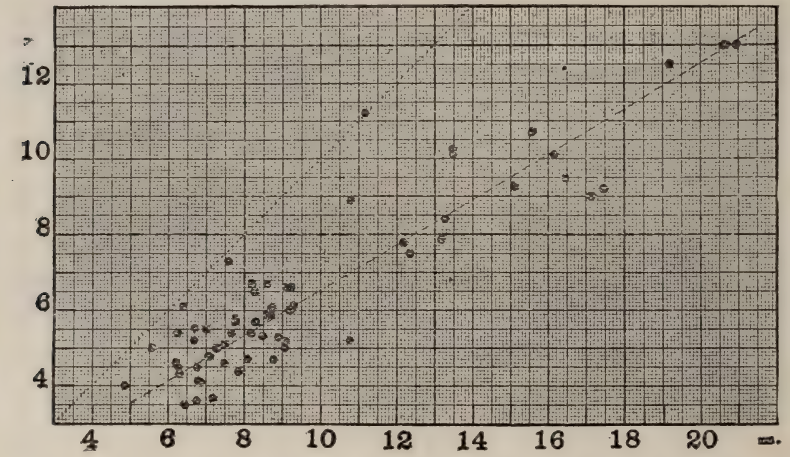

FIG. 1. Relation between length of male *Gammarus locusta* and length of female; measurements of 62 pairs. The dotted line shows curve of relation, if male and female were of equal size in each pair. The line drawn through the plotted points shows how the actual assortment depends in part on the intrinsically smaller average size of the females.

It is possible that this phenomenon, in varying degrees, is of wide occurrence among animals. As further evidence there is here presented a study of naturally occurring pairs of *Gammarus locusta* (Linn.), a small salt-water crustacean. It is possible to study size relations of the members of breeding pairs, because the male carries the female about with him for a considerable time. The formation of such pairs is by the usual sex-recognition methods of the crustacea. It is agreed by those who have studied sex-recognition in typical crustaceans that it is a purely mechanical affair,[2] and this was observed to be true in *Gammarus*. The male shows certain strong clasping reflexes, and itself resists being clasped. The female, however, is usually passive when clasped.

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[1] Crozier, W. J., *Jour. Exper. Zoöl.*, 1918, xxvii, 247; *Amer. Nat.*, 1920, v, 182.

[2] Holmes, S. J., *Biol. Bull:*, 1903, v. 288; *ibid.*, 1909, xvi, 313. Andrews, E. A., *Jour. Exper. Zoöl.*, 1910, ix, 235.

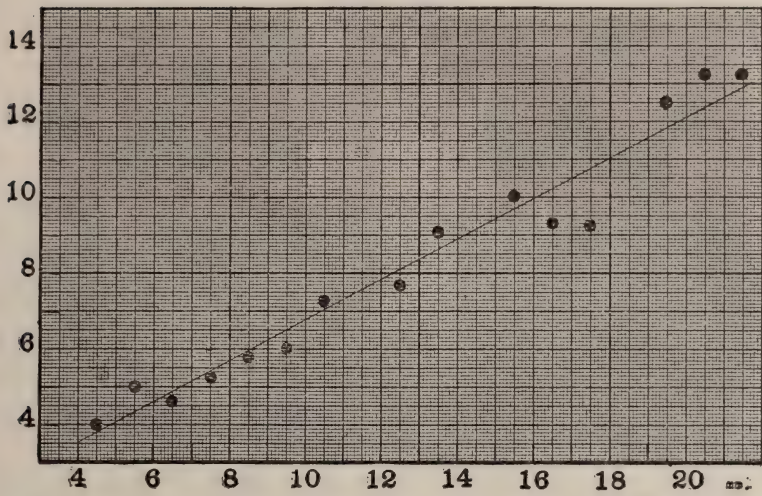

FIG. 2. Regression plot; ordinates are the main lengths of the mates of males in the corresponding length-classes. The index of correlation (length of male : length of mate) is $r = 0.804 \pm 0.030$.

With the help of a student, Mr. Connemacher, pairs of *Gammarus locusta* were collected on the shore of Raritan Bay, on Staten Island. The length of each of the members of each pair was measured. The results show a high degree of selective coupling on the basis of length. The figures bring this out clearly. Large individuals tend to mate with large, and small with small. This appears to be entirely automatic.

The upper limit of length of the female with which any given male will mate seems to be determined by his ability to clasp and hold her.[1] The lower limit is much more elastic, but is nevertheless a limit, apparently also determined by mechanical features of the clasping process.

These animals can be bred in the laboratory, and it should therefore be possible to discover the result or lack of result of the selective coupling.

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[1] Holmes, *loc. cit.* (1903).

### 155 (1902)

## Symmetry of heliotropic orientation in slugs.

By **W. J. CROZIER** and **W. H. COLE.**

[*From the Zoölogical Laboratories, Rutgers College, New Brunswick, N. J., and Lake Forest College, Lake Forest, Ill.*]

The creeping of the slug *Limax maximus* shows no apparent neuromuscular asymmetry. But in heliotropic orientation there is chiefly involved the parietal musculature, where differential activity might conceivably be associated with the fundamental torsion of the body of the gasteropod. The point has been studied in relation to the occurence of "trial movements" at the beginning of the act of orientation, and has a special significance also for the analysis of "circus movements."

Experiment shows that a previously quiescent, dark-adapted *Limax*, illuminated from in front, turns with equal frequency to the right or to the left. *Limax* is negatively heliotropic, for all intensities used in these experiments. A similar equal frequency of turns to right and to left is found under non-stimulating (red) light. It would be incorrect to speak of these movements as "trial movements," for in a series of 142 observations it was found that in 122 the direction of orientation coincided with that of the first indication of turning; only in 20 did the side definitively contracted differ from that first contracted (8 right; 12 left).

The relative tensions developed by the two sides of the body, when right or left is the particular side toward which orientation is made, may be decided by comparing the rates of orientation in the two cases. Making allowance for the fact that the rate of orientation varies inversely with the size of individual, by comparing only "right" and "left" records for the same individuals, it is found that there is no sensible muscular asymmetry to be considered in the measurement of circus movements. Asymmetry of this type reported for some shelled gasteropods may be due to the mechanical effect of the shell's weight.

### 156 (1903)

## The relation between the accumulation of globulins and the appearance of agglutinins in the blood of newborn calves.

By **MARION L. ORCUTT** and **PAUL E. HOWE.**

[*From the Rockefeller Institute, Princeton, N. J.*]

A study has been made to determine the protein fractions of colostrum and of serum which carry the agglutinins produced against *Bacillus abortus.* Data have been obtained indicating that the agglutinins from serum and colostrum are almost completely precipitated with the globulin fraction separating at 14.2 per cent. of sodium sulfate; complete precipitation occurs at 16.4 per cent. of sodium sulfate. That such a separation is not the result of adsorption due to precipitation is shown in experiments in which casein was removed from colostrum with acetic acid; the casein removed did not have agglutinins associated with it but agglutinins did appear with the subsequent fractions.

The coincident appearance of the globulin fractions and of the agglutinins in the blood of newborn calves has been shown by direct comparison of the appearance of the proteins and the agglutinins. Where globulins precipitated by concentrations of sodium sulfate less than 17.4 per cent. have not been absorbed agglutinins have not been absorbed, on the other hand the absorption of globulins has been demonstrated in cases where the agglutinins for *B. abortus* were not present. A case in which agglutinins have been presented to a newborn animal without the associated globulins, if such is possible, has not been considered.

The evidence presented is associated with the observations of Little and Orcutt[1] and of Howe.[2] In the first case it was shown that the blood of newborn calves before ingesting colostrum did not contain agglutinins against *B. abortus* but that after the ingestion of agglutinin-containing colostrum the blood had a titer related to the agglutinin content of the colostrum. The observation of Howe demonstrated that the blood serum of a newborn calf does not contain appreciable quantities of proteins precipitable by concentrations of sodium sulfate equal to or less than 17.4 per cent. of the anhydrous salt, euglobulin and pseudoglobulin I. After the ingestion of colostrum containing these globulins relatively large quantities of the proteins appeared in the serum. Subsequent data have shown that there is evidence of absorption of the proteins within 3 to 4 hours and a very marked accumulation of the proteins 6 hours after receiving colostrum. Furthermore, we have one case in which both the agglutinins (Little) and the globulins decrease in amount with increasing age. The time and conditions of the formation of the globulins is being studied. The demonstration that the agglutinins are associated with the globulin fractions of the proteins of blood and colostrum and their absorption by young animals tends to support the idea of direct absorption into the blood of newborn calves of certain protein fractions present in colostrum.

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[1] Little, R. B., and Orcutt, M. L., *J. Exp. Med.*, 1922, xxxv, 161.

[2] Howe, Paul E., *J. Biol. Chem.*, 1921, xlix, 115.

The conception of a direct absorption of agglutinins and protein by the young animals based upon biological reactions has appeared repeatedly in the literature relating to the transmission of immunity. The absence of a substance reacting with colostrum antiserum in the blood of newborn calves which is acquired after the ingestion of colostrum has been demonstrated by Langer.[1] The acquirement of relatively large quantities of particular protein fractions by the newborn and the association of the agglutinins with these fractions we believe to be a new demonstration.

## 156 (1903)

## The effect of saline purgatives on the absorption of other drugs.

By DAVID I. MACHT and E. M. FINESILVER.

[*From the Pharmacological Laboratory, Johns Hopkins University, Baltimore, Md.*

It is well known that the pharmacodynamics of saline purgatives consists chiefly in the poor absorbability of certain ions such as those of magnesium, sulphate, phosphate, etc., and the accumulation of fluid in the intestinal canal through the osmotic action of the unabsorbed salt which, instead of being absorbed, actually draws fluid into the intestinal lumen. This peculiar phenomenon led the author to inquire into what effect the administration of such purgatives may have upon the absorption of other drugs given by mouth simultaneously or a little after the laxative. Accordingly experiments were first made with phenolsulphonphthalein. This drug, as is well known, is rapidly absorbed, whether given by injection or by the stomach, and is equally as rapidly excreted by the kidneys. Several dogs were given a given quantity of a solution of phenolsuphonphthalein by stomach tube and the amount excreted in the urine at the end of one and two hours was determined quantitatively by the colorimetric method. Several days later after the drug had been completely excreted, the same amount of dye with the same amount of fluid was administered to the same animals by the same method, with the exception that the dye was this time mixed with a solution of sodium sulphate (5 per cent.) instead of plain water. The excretion of the dye by the kidneys was studied at the end of each hour as in the first series of experiments. It was found that the excretion of the phenolsulphonphthalein was markedly delayed by the simultaneous or previous administration of sodium sulphate. The same was true of magnesium sulphate and other saline purgatives, but no such effect was produced by the administration of other cathartics of a non-saline character such as castor oil or cascara sagrada.

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[1] Langer, *Verhandl. d. Gesellsch. f. Kinderheilk.*, 1907, xxiv, 70.

To study the mechanism of the above phenomenon more in detail, experiments were then made on cats. The animals were anesthetized, laparotomy was performed and two loops of intestine of exactly the same length were tied off, in some experiments in the same animal, and in other experiments in two separate animals. Into one loop 1 c.c. of standard phenolsulphonphthalein solution mixed with a given volume of water was injected. Into the other loop exactly the same amount of dye was mixed with exactly the same volume of sodium-sulphate solution. The intestines were replaced in the abdominal cavity and the abdomen closed. At the end of an hour the animals were killed and each loop of the intestine was cut out and its contents carefully measured. It was found that in the control loops, that is, the loops containing a solution of the dye in water, much of the fluid was absorbed and the amount of dye remaining in the contents of that loop plus any dye that could be rinsed out of the intestinal mucosa examined colorimetrically indicated a marked absorption (in some cases as high as 60 or more per cent.) at the end of an hour. When the contents of the loops containing the dye with the saline solution were examined it was noted at once that the volume of fluid in the loop was greatly increased due to osmotic drawing of fluid into the lumen. A quantitative determination of the dye content in these loops showed that over 90 per cent. of the dye had been unabsorbed at the end of an hour. On dipping the mucosa of each loop in weak alkali a striking picture was obtained. Whereas the normal or control loops became intensely red indicating the passage of dye into the villi and the circulation, the appearance of the other loop (the "saline loop") was very pale and showed very little red color, thus indicating the very poor absorption of the dye.

Following the above experiments with phenolsulphonphthalein, an extensive investigation was undertaken on the effects of saline purgatives on the absorption of a large number of drugs. Details will be published in the full paper. In this place suffice it to say that representatives of various classes of drugs were tested. Among these may be mentioned digitalis, various antipyretics such as salicylates, acetanilid, and antipyrine, etc., a number of salts such as urotropin, iodides, etc., a number of opiates, various alkaloids, bichloride of mercury and other substances. It was found that almost every kind of drug that was examined was delayed more or less in its absorption by the previous and even by the simultaneous administration of sodium sulphate, magnesium sulphate and other saline laxatives. These effects were determined in some cases by physiological tests and in other cases by chemical examination. In case of non-toxic drugs the observations were corroborated by tests on human volunteers. The studies on the influence of saline purgatives on the phenolsulphonphthalein kidney function test are to appear in a forthcoming number of the *Journal of Urology*. A study of other classes of drugs in this respect will appear in the Bulletin of the Johns Hopkins Hospital.

158 (1905)

### Chemical stimulation of the nerve cord of Cambarus clarkii.

By **A. R. MOORE.**

[*From the Physiological Laboratory of Rutgers College, New Brunswick, N. J.*]

A preparation of the central nervous system of crayfish was made by decapitating the animal and uncovering the nerve cord of the posterior abdominal segments. Application of a stimulating substance to this portion of the cord resulted in convulsive movements of the thoracic appendages. Stimulation was obtained from application of excitants of the first class, $BaCl_2$, KCl, $Na_3$ citrate, in concentrations isosmotic with the animal's blood. Tetraethylammonium chloride in M/64 concentration acted as a strong excitant. Of the excitants of the second class, camphor, strychnine, atropine, picrotoxin, nicotine, caffeine and phenol were active. Creatine alone showed no effect. The central nervous system of *Cambarus* therefore differs from that of *Lumbricus*[1] in being sensitive to the action of nicotine, phenol and caffeine. The two forms are alike in being insensitive to creatine, and in the fact that the latent period for chemical stimulation is very short, less than a minute.

159 (1906)

### The respiratory rate of the nerve cord of Cambarus clarkii.

By **A. R. MOORE.**

[*From the Physiological Laboratory of Rutgers College, New Brunswick, N. J.*]

The rate of production of $CO_2$ of the nerve cord of *Cambarus* was determined by means of the colorimetric method previously described.[2] The nerve cord was removed entirely freed from other tissue. Stimulation due to cutting was only momentary. It could be shown, by leaving the cord attached at the anterior

[1] Moore, A. R., *J. Gen. Physiol.*, 1921, iv, 29.

[2] Moore, A. R., *J. Gen. Physiol.*, 1918–19, i, 613.

end, that irritability was retained by the posterior dissected portion of the cord for more than 45 minutes. Since it is possible to complete a respiration experiment in 20 minutes, the tissue must have been irritable throughout each experiment.

The procedure consisted first in determining the rate of $CO_2$ output for the resting cord. The effect of activity on the respiratory rate was found by suspending the tissue in the indicator tube on platinum electrodes which were passed through a paraffined stopper, and then stimulating the cord with induction shocks while the reading was being made. The current used for this purpose was not sufficient of itself to cause any change in the tint of the indicator, no matter how long continued. A third reading was now made with the tissue at rest. The relative rates of respiration in the three cases were determined by calculating the second and third readings as per cents. of the first. Averages of ten experiments made on the nerve cords of as many different animals yielded the following result: Resting 100 per cent., stimulated 89 per cent., resting 86 per cent. Electrical stimulation, therefore, not only did not increase the rate of $CO_2$ production of the nerve cord of *Cambarus*, but failed to interrupt the normal fall in rate. The question may thus rightly be raised whether functional activity of the cells of the central nervous system of the cryfish is accompanied by increased metabolic activity.

### 160 (1907)

## The value of intratracheal route of immunization with pneumococcus.

By **J. BRONFENBRENNER** and **E. KNIGHTS.**

[*From The Department of Preventive Medicine and Hygiene, Harvard Medical School, Boston, Mass.*]

The ease which with gases diffuse through the respiratory mucosa is well known and is widely utilized in the practice of anesthesia. The absorption from the trachea, however, is not limited to gases. Thus, Mayer[1] found potassium ferrocyanide in the blood two to five minutes after its introduction into the trachea of animals. Moreover, even such substances which do not easily diffuse through other tissues may sometimes readily pass through the mucosa of the respiratory system. Thus, for instance, Colin introduced intratracheally 10 c.c. of 1 per cent. solution of curare (which is not absorbed from the intestine) killing the dog in 15 minutes. The rate at which fluids may be absorbed from the trachea is remarkable. Colin[2] describes an experiment in which he introduced intratracheally into a tracheotomized horse a continuous stream of warm (30°–35°) water at a rate of six liters per hour for 3½ hours in succession without causing any noticeable discomfort to the horse. When at the end of the experiment the horse was sacrificed the observer could not detect any water in the trachea or bronchi, all water having been thoroughly absorbed.

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[1] Muller, Manual de physiologie, Paris, 1851, p. 186.

In spite of this remarkable power of absorption, trachea was not generally employed as the route for the parenteral introduction of foreign substances in the experimental work until recently when Besredka[3] called attention to the fact that trachea constitutes as good and perhaps even a better site of introduction of antigen into the experimental animals than any other employed. During the summer of 1920 one of us had the privilege of personal acquaintance with the work of Besredka and it is this experience that suggested the possibility of utilizing the tracheal route for the purpose of production of immunity to pneumococcus. This investigation was undertaken with the hope that intratracheal introduction of pneumococcus antigen might be particularly advantageous in view of the fact that it suggested the possibility of increasing the local resistance of the tissues of the respiratory system to pneumococcus in addition to creating the state of humoral immunity. The experiments which will be reported later will bear on the question of successful production of increased local tissue-resistance. In this paper we wish to report on the relative value of *intratracheal* route for parenteral introduction of pneumococcus antigen as compared with subcutaneous, intravenous and intrapleural routes and as measured by the concentration of circu-

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[1] Colin, Traite de physiologie comparee des animeau, Paris, 1873, t. 88, p. 112.

[2] Colin, loc. cit., pages 109–110.

[3] Besredka, *Ann. Inst. Pasteur*, 1920, xxxiv, 51 and 361.

lating antibodies in animals treated respectively by the four above-mentioned methods. A series of normal rabbits were divided into four groups of two animals each and immunized by the repeated introduction of a suspension of Type I pneumococcus (heated for 30 minutes at 60° C.) by the four above-mentioned routes. In all the animals received 9 injections each, over a period from October 1 to November 17. Special care was taken to maintain the uniformity of the amount of bacterial protein injected in respective series. Ten days after the last injection the blood of all the animals was tested for its bactericidal power, for its opsonic power and for its power to protect mice against infection with virulent culture.

*Bactericidal power* was tested by the technique of Heist and Solis-Cohen.[1] Bacterial suspension containing 1,600,000 pneumococci per cubic centimeter was diluted as indicated on the chart below.

| Rabbit No. | Route of Immunization. | Dilutions of Bacterial Suspension. | | |
|---|---|---|---|---|
| | | 2:25 | 1:125 | 1:625 |
| 328 | Intravenous | ++ | ++ | ++ |
| 329 | Intravenous | + | + | + |
| 330 | Intratracheal | − | − | − |
| 331 | Intratracheal | + | − | − |
| 332 | Subcutaneous | +++ | ++ | ++ |
| 333 | Subcutaneous | ++ | + | + |
| 334 | Intrapleural | ++ | + | − |
| 335 | Intrapleural | ++ | − | + |
| 336 | Normal controls | +++ | +++ | +++ |
| 337 | | ++ | ++ | +++ |

The bactericidal power of the blood is of course inversely proportional to the extent of growth.

*Opsonizing power* of the respective sera was determined by using mouse leucocytes and was expressed in terms of percentage relation of the number of leucocytes in the state of active phagocytosis to the total number of leucocytes counted.

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[1] Heist, George D. and Cohen-Solis, Solomon, *Journal of Immunology*, 1919, iv, No. 4.

| | |
|---|---|
| +++ | Very good growth. |
| ++ | Medium growth. |
| + | Poor growth. |
| − | No growth. |

The relative opsonizing power of various sera was found to be as follows:

| Rabbit No. | Route of Immunization. | |
|---|---|---|
| 328 | Intravenous | 2% to 8% |
| 329 | Intravenous | |
| 330 | Intratracheal | 10% to 20% |
| 331 | Intratracheal | |
| 332 | Subcutaneous | 2% to 4% |
| 333 | Subcutaneous | |
| 334 | Intrapleural | 10% to 20% |
| 335 | Intrapleural | |
| 336 | Normal (control) | below 1% |
| 337 | Normal (control) | |

In the test for protection three series of mice received intraperitoneally 1/2 c.c. of the respective sera each. Immediately following the serum mice of respective series received intraperitoneally virulent culture in the amount representing from 12,500 to 50,000 M.L.D.

| Route of Immunization. | Number of M.L.D. of Pneumococcus. | | |
|---|---|---|---|
| | 12,500 | 25,000 | 50,000 |
| Intravenous | living | living | + 24 hrs. |
| Intratracheal | living | living | living |
| Subcutaneous | + 24 hrs. | + 24 hrs. | + 24 hrs. |
| Intrapleural | living | living | living |
| Normal (control) | + 24 hrs. | + 24 hrs. | + 24 hrs. |

### 161 (1908)

## Racial variations in Blepharisma undulans.

By **LORANDE LOSS WOODRUFF** and **HOPE SPENCER.**

[*From the Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.*]

Studies on *Blepharisma undulans* and *Blepharisma lateritia* Stein, have emphasized the impossibility or great difficulty of observing the micronuclei, and also the inevitable death of exconjugants without dividing. Thus Bütschli[1] was unable to identify micronuclei in vegetative specimens of *B. lateritia* though he found them in conjugants. Exconjugants invariably died. Calkins[2] did not find micronuclei in non-dividing *B. undulans*, but discovered them during division within the macronuclear membrane, and described their emergence and activities during conjugation. He likewise was unsuccessful in obtaining a single viable exconjugant.

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[1] Bütschli, *Abhandl. d. Senckenb. naturf. Gesellsch. Frankfurt a/M.*, 1876, Bd. x.

[2] Calkins, *Journ. Morph.*, 1912, xxiii.

During the past five months we have had under observation a pedigree culture of *Blepharisma undulans* which emphasizes racial differences within the species. The animals of this race possess from four to fourteen relatively conspicuous micronuclei, all of which are free in the cytoplasm during every stage of the life of the cell; and all the exconjugants thus far secured proved to be viable.

The main culture has attained one hundred and fifty generations to date, an average of about one division per day, in the standard beef extract culture medium used in this laboratory. At intervals, epidemics of conjugation have appeared. Exconjugants have been isolated and new lines established from their progeny. Studies are in progress similar to those which have been conducted on *Spathidiums*.[1]

The wide experience of such observers as Bütschli and Calkins precludes the assumption that they were in error in regard to the micronuclear condition of the animals which they studied and the only reasonable conclusion is that there are races of *Blepharisma* which differ in regard to the vegetative position of the micronuclei; in some it is intramacronuclear and others extramacronuclear—the present pedigree race of *Blepharisma undulans* representing the latter and therefore agreeing with the marine species, *Blepharisma clarissima*, as described by Anigstein.[2]

The results of all previous workers are consistent in regard to to the non-viability of exconjugants. Whether the intramacronuclear position of the micronuclei bears any causal relation to non-viability it is impossible to decide. Certainly there are no obvious differences between the chief nuclear phenomena described by Calkins in his non-viable conjugants and those which occur in the viable conjugants of the pedigree race which we are conducting.

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[1] Woodruff and Spencer, PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 180, 240 303.

[2] Anigstein, *Archiv. f. Protistenk.*, 1912, Bd. xxiv.

162 (1909)

## Endomixis and encystment in Spathidium spathula.

By **E. LUCILE MOORE** (by invitation).

[*From the Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.*]

In a pedigree culture of *Spathidium spathula* there has been no indication of endomixis at any time during active vegetative life, and experiments were undertaken to determine the possibility of inducing an independent reorganization process in conjugating individuals by preventing nuclear interchange. Since conjugation in this race produces a significant increase in the division rate of the majority of exconjugant lines,[1] it was expected that a similar physiological effect would follow endomixis. Pairs in an early stage of fusion were separated by spurting from a pipette, and the division rates of lines derived from such split-conjugants were compared with those of normal exconjugant and non-conjugant lines.

Of seventeen exconjugant lines, thirteen produced an average of 7.3 generations more than the parent lines during the first fifteen days. Of thirty-nine split-conjugant lines, however, all but four produced essentially the same number of generations as the parent lines, indicating, apparently, that endomixis had not occurred. Additional evidence further supported this view.

Endomixis, however, has been observed in this race during encystment. Cytological study of encysted individuals at various stages in the process has revealed the fragmentation and resorption of macronuclear material, the persistence of micronuclei, and the formation of macronuclear anlagen. The physiological significance of endomixis during encystment has not been determined.

The complete paper will appear in the *Journal of Experimental Zoölogy*.

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[1] L. L. Woodruff and Hope Spencer, PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 240.

### 163 (1910)

## The existence of an attracting stimulus in the development of the central nervous system.

By DAVENPORT HOOKER.

[*From the Department of Anatomy, School of Medicine, University of Pittsburgh, Pittsburgh, Pa.*]

Studies on the spinal cord of amphibian embryos have demonstrated the remarkable regenerative powers of the central nervous system in these forms. The cord may be completely severed in frog embryos and tadpoles up to the time of metamorphosis and will restore anatomical and physiological continuity in a high percentage of cases. In the earlier stages, the removal of a segment of the cord one or two myotomes in length is followed by complete reëstablishment of anatomical continuity in most cases. Where restoration of anatomical continuity fails for various reasons, the regenerated or developing nerve fibers have grown toward the opposite cut end of the cord, sometimes by unusually tortuous routes.

A series of experiments on young embryos in which a segment of the spinal cord was removed, rotated on its long axis and reimplanted, gives further indication of the existence of an attracting stimulus as a factor in reëstablishing the continuity of the cord.

These experiments indicate that it is possible to obtain reestablishment of anatomical continuity between a portion of the cord in its normal position and a segment which has been rotated on its long axis through various arcs up to complete inversion (180° rotation). The restoration of physiological continuity has not been conclusively demonstrated, though there is now much evidence that it has occurred. Better healing is obtained when the segment is rotated up to 90° than when it is rotated more. When rotated 90°, there is a tendency for the regenerated tissue closing the cord wound to rotate through the quarter turn of a spiral to link up the like fiber tracts. This same tendency is present in rotations of from 90° to 135°, but is not so clearly demonstrable. The rotation of the regenerated area of the cord is caused by the spiral course taken by the developing nerve fibers.

These experiments demonstrate the existence of a definite stimulus attracting developing nerve fibers within the spinal cord.

The explanatory theories of Cajal, Kappers and his students and Child seem inadequate. It is hoped that further work on this problem, now under way, will give a decisive answer to this question.

### 164 (1911)

## Methods of estimating the activity and intelligence of normal and thyroidectomized sheep.

By H. S. LIDDELL (by invitation).

[*From the Physiological Laboratory, Cornell University Medical College, Ithaca, N. Y.*]

The attempt has been made to devise methods for investigating the activity and learning capacity of sheep following the extirpation of the thyroid glands. Their intelligence was tested by their ability to learn a simple labyrinth and the twin of each pair making the better record was thyroidectomized by Dr. Simpson. Later, when the operated lambs showed clearly the stigmata of cretinism the pairs were again tested in order, if possible, to demonstrate any influence of hypothyroidism on the intelligence of the operated lamb when compared with its normal twin. A labyrinth with a single cul de sac has been constructed in which, by a system of gates, the position of the cul de sac can be reversed. This arrangement makes possible the presentation of a number of problems so that the tests on a pair of lambs can be continued as long as may be necessary to determine the effect of the extirpation of the thyroid glands on the ability of the operated lamb to profit by experience.

The effect of thyroidectomy on the activity of the lambs was also sought for by comparison of each cretin lamb with its normal twin. It was found practicable to measure the activity of the sheep by attaching a pedometer to the fore leg and calibrating the instrument to measure the number of steps which the animal takes instead of the distance it covers. The pedometer is sufficiently delicate as an indicator of the animal's spontaneous activity to demonstrate lethargy if it occurs following throidectomy. An apparatus has been constructed by which the cretin's capacity for muscular exertion can be compared with that of the normal lamb. It is essentially an inclined plane the angle of which can be altered at will. It is not difficult to induce the sheep to ascend the incline and so strong is the flock instinct that a sheep too weak to follow the others will continue its attempts until exhausted.

### 165 (1912)

## The deleterious effect of sodium citrate on the blood with particular reference to the H-ion concentration.

By **RALPH R. MELLON, WILLARD S. HASTINGS** and **GERTRUDE M. CASEY.**

[*From the Highland Hospital Laboratories, Rochester, New York.*]

Experiments were undertaken to determine whether the effects recently reported by Unger[1] to result from the action of sodium citrate on the blood when added in the proportions used in transfusion might not be related in some way to the hydrogen-ion concentration of the solution used. These effects, as given by Unger, included the formation of a substance derived from the stroma of the red cells which is anticomplementary in the Wassermann reaction, the red cells being at the same time rendered more fragile, together with a direct interference with the action of complement, a practical destruction of the phagocytic activity of the leucocytes, and a reduction in the effect of opsonin.

Three sodium citrate solutions (2 per cent.) having $P_H$ values of 4.1, 7.25 and 9.56 were added to blood in ratios of 1 : 2 and 1 : 9. With but an occasional exception the citrated plasmas thus obtained showed no anticomplementary power. With both whole blood and plasma a varying amount of precipitate usually appeared, which increased on inactivation. It was not present in citrated serum. The citrated extracts of washed red cells were not anticomplementary. Repeated tests of the citrated blood for red-cell fragility were negative, as indeed were the phagocytic indices of leucocytes exposed to citrate. In the main our results were diametrically opposite to those reported, regardless of the $P_H$ of the solution used. These results indicate that the reactions attending the use of citrate must be sought by other criteria. They may be associated with the disturbance of equilibrium causing the precipitate above mentioned.

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[1] Unger, L. J., *J. A. M. A.*, 1921, lxxvii, 2107.

## ABSTRACT OF THE COMMUNICATIONS, PACIFIC COAST BRANCH.

### Thirty-third meeting.

*Berkeley, California, April 12, 1922.*

166 (1913)

### The antigenic properties of red-cell globulin.[1]

By **CARL L. A. SCHMIDT** and **D. E. DEMENT.**

[*From the Department of Biochemistry and Pharmacology, University of California, Berkeley, Cal.*]

Three theories have recently been advanced relative to the nature of the antigen which, on repeated injection of foreign red cells, gives rise to a specific hemolytic sensitizer in the blood stream of the immunized animal. Balls and Korns[2] have come to the conclusion that the antigen is contained in the stroma of the red cell and that it is neither a globulin nor an albumin but probably a nucleoprotein. While the presence of nucleic-acid residues in non-nucleated red cells cannot be denied, the careful work of Bloor[3] indicates that within the limits of experimental error of his method the presence of nucleoprotein in red cells appears doubtful. The experiments of Wooldridge[4] on the constituents of the stroma of red cells shows that although a protein combined with a molecule containing phosphorus (this may be lecithin) is present in small quantities, the greater part of the protein fraction consists of paraglobulin.

While it is not to be denied that immunization with stroma does lead to the appearance of a hemolytic sensitizer, the experiments of Ford and Halsey[1] as well as those of Bennett and Schmidt[2] nevertheless indicate that it is also possible to obtain a hemolytic sensitizer by immunization with the water-soluble portion of red cells. Moreover, the findings of Balls and Korns that the filtrate obtained by passing a solution of hemolyzed red cells through a porcelain filter does not bind hemolytic sensitizer do not appear to us as conclusive evidence that the antigen is contained wholly in the stroma. It is a well-known fact that the first portion of the filtrate obtained on passing a solution of proteins such as serum through a porcelain filter invariably shows a loss of protein and that the latter portions of the filtrate are relatively richer in protein than the first. The experiments of Muir[3] with the water-soluble portion of red cells clearly indicate that the first portion of the filtrate is unable to bind sensitizer while the latter portions can unite with increasing amounts.

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[1] Aided in part by a grant from the Research Board of the University.

[2] Balls, A. K., and Korns, J. R., *Jour. of Immunology*, 1918, iii, 375.

[3] Bloor, W. R., *Jour. Biol. Chem.*, 1918, xxxvi, 49.

[4] Wooldridge, L., *Arch. f. Anat. u. Physiol.* (Physiol. Abt.), 1881, p. 387.

Vedder[4] dissolved the stroma obtained from human red cells in dilute alkali, neutralized the solution with acetic acid and filtered off the protein precipitate. Rabbits were immunized with both this fraction and the protein contained in the filtrate. The latter, which Vedder believes to be an albumin, gave rise to the hemolytic sensitizer for human cells while no antibodies were obtained by immunization with the acetic acid precipitate.

Bennett and Schmidt[2] carried out experiments with the $CO_2$-globulin isolated from a solution of the constituents of ox red cells after removal of stroma by centrifuging and filtration. Rabbits were immunized with this protein and a specific sensitizer and an agglutinin for the homologous red cells were obtained. Since the experiments were carried out with the red cells of only one species and since the experiments of Vedder indicate that possibly in the red cells of other species a protein other than the $CO_2$-globulin may be the antigen concerned in the production of hemolysis, it appeared to us desirable to carry out experiments with the globulins obtained from the red cells of several other species.

Antigens were prepared from the red cells of the sheep, the pig and man in accordance with the method described by Bennett and Schmidt. Rabbits were immunized by intraperitoneal injections of these respective antigens at definite intervals of time and tests for the presence of hemolytic sensitizer and of agglutinin in the serum of the injected animals were carried out as in the experiment with the globulin from ox cells. The data follows:

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[1] Ford, W. W., and Halsey, J. T., *Jour. of Medical Research*, 1904, xi, 403.

[2] Bennett, C. B., and Schmidt, C. L. A., *Jour. of Immunology*, 1919, iv, 29.

[3] Muir, R., "Studies on Immunity," London, 1909, p. 129.

[4] Vedder, E. B., *Jour. of Immunology*, 1919, iv, 141.

Rabbit No. 44 was given a total of 75 c.c. of a suspension of $CO_2$-globulin prepared from sheep cells. This corresponds to 35 c.c. of whole sheep blood and contained approximately 20 mgs. of nitrogen. The limit of the hemolytic titer was found to be 0.4 c.c. of 1 : 6,000. The serum showed marked agglutinative properties.

Rabbit No. 45 received the same amount of sheep-cell globulin as No. 44. The limit of the hemolytic titer was 0.2 c.c. of 1 : 6,000 and the sheep cells were markedly agglutinated by this serum.

Rabbit No. 40 was given a total dosage of 175 c.c. of $CO_2$-globulin prepared from pig cells, an amount which corresponds to 80 c.c. of whole blood. Its content of nitrogen was 60 mgs. The limit of the hemolytic titer was found to be 0.1 c.c. of 1 : 250 and this was also the limit of agglutination.

Rabbit No. 42 received the same amount of pig-cell globulin as No. 40. The limit of the hemolytic titer was found to be 0.1 c.c. of 1 : 250 and the highest dilution of serum which agglutinated the pig cells was 0.3 c.c. of 1 : 1,250.

Rabbit No. 36 was given a total dosage of 210 c.c. of human red-cell globulin. This corresponds to 50 c.c. of whole blood and contained 23 mgs. of nitrogen. The hemolytic titer of the serum was found to be 0.2 c.c of 1 : 50 and a suspension of red cells were agglutinated in a serum dilution of 1 : 250.

Rabbit No. 37 received the same dosage of $CO_2$-globulin from human red cells as animal No. 36. The hemolytic titer of the serum was found to be 0.1 c.c. of 1 : 10 and the highest dilution of serum which agglutinated red cells was 0.1 c.c. of 1 : 1,250.

Rabbit No. 38 received the same dosage of human $CO_2$-globulin as the two previous animals. The hemolytic titer was found to be 0.4 c.c. of 1 : 50 and the limit of agglutination was 0.1 c.c. of 1 : 1,250. It was not found possible to raise the titer of the last three sera. However it is frequently found that only comparatively low titer sera are obtained when rabbits are immunized with human red cells.

These experiments definitely indicate that immunization with the $CO_2$-globulin prepared from the water-soluble portion of the red cells of the sheep, the pig and man leads to the appearance, in the blood stream of the treated animal, of a specific hemolytic sensitizer and of an agglutinin. It appears possible, although our experiments are incomplete on this subject, that the globulin from the water-soluble portion of the red cell is closely related to one of the proteins contained in the stroma, since by immunization with either of these antigens, the same antibody is obtained.

### 167 (1914)

## The separation of the hexone bases from a protein hydrolysate by electrolysis.[1]

By G. L. FOSTER and CARL L. A. SCHMIDT.

[*From the Department of Biochemistry and Pharmacology of the University of California, Berkley, Cal.*]

In a previous communication describing a method for the preparation of glutamic acid, the need of developing cheaper methods for the production of amino acids in quantity was pointed out. This is especially true with respect to the amino acids arginin, histidin and lysin. The cost of reagents and the labor required for the preparation of these amino acids prohibits experimental work in which large quantities of these substances are required.

Some years ago Ikeda and Suzuki[2] described a method for separating certain fractions of the products of protein hydrolysis. Their method has apparently not come into general use and experimental data are not available. On passing direct current through a solution of the protein cleavage products, which is placed in the center of a three-compartment cell, the amino acids are separated into three fractions consisting of (*a*) the amino

[1] Aided by a grant from the Research Board of the University.

[2] Ikeda, K., and Suzuki, S., U. S. Patent No. 1015891, Jan. 30, 1912.

acids which are predominantly acid, including aspartic and glutamic acid, which migrate to the anode, (*b*) the basic amino acids which include arginin, histidin and lysin, and which wander to the cathode, and (*c*) the remaining amino acids, which on account of the fact that their acid properties are about equally balanced by their basic properties, remain in the center compartment.

Since this method eliminates the reagents which, in other methods, are required in order to separate the hexone bases, experimental work was carried out on a laboratory scale to determine the general applicability of this method to the isolation of the basic amino acids.

The electrolytic cell consists of a rectangular wooden box 3 × 6 × 4.5 inches which was cut into three approximately equal vertical sections. The membranes separating the compartments consist of strips of linen cloth which were coated with gelatin by immersion in a 30 per cent. solution of this substance and the gelatin was subsequently fixed by allowing the strips to remain in formalin over night. After placing the membranes in position, the three parts of the cell are clamped by means of bolts. For the purposes of water-proofing, the cell was painted with asphalt. Thin sheets of carbon were used instead of the iron electrodes recommended by Ikeda and Suzuki. The latter were found to dissolve at the anode and $Fe(OH)_3$ to precipitate in the cathode solution, necessitating its subsequent removal. The fluid in the center compartment was kept at the $P_H$ indicated in the table by the addition of small quantities of $Ba(OH)_2$ at frequent intervals. Similarly the reaction of the cathode liquor was kept approximately neutral by the occasional addition of $H_2SO_4$. The temperature of the solutions was kept below 35° C. by circulating a stream of water through a test tube which was placed in the center compartment, and by continuous agitation of the solution. Distilled water was placed in the anode and cathode compartments. Electrolysis was effected by passing 1.5 amperes of the 110-volt circuit through the cell.

The experiments were carried out with gelatin since this substance is a convenient source for arginin. The protein was hydrolyzed with the aid of 30 per cent. $H_2SO_4$ as suggested by Dakin,[3] the acid was neutralized by addition of $Ba(OH)_2$ in slight excess and the ammonia was removed by blowing air through the solution for several days. The electrolysis, which requires approximately three hours, is continued until a specimen of the fluid from the center compartment, on addition of phosphotungstic acid, gives no precipitate. The fluid in the cathode solution invariably contained approximately 15 per cent. of non-basic nitrogen. A small quantity of amino acids other than the hexone bases are carried over into the cathode compartment by cataphoresis. On reëlectrolysis of the cathode solution the non-basic nitrogen was reduced to an indeterminable quantity.

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[3] Dakin, H. D., *J. Biol. Chem.*, 1920, xliv, 499.

The table shows the results which were obtained in a number of experiments and indicates the applicability of the method for the separation of the basic group of amino acids. Of particular interest is the observation that when the reaction of the gelatin hydrolysate is more alkaline than $P_H$ 7.5, histidin is not carried over into the cathode compartment while $P_H$ 5.5 analysis of the cathode solution shows the presence of the three basic amino acids in approximately the same proportions as in the original hydrolysate. This is not unexpected when it is recollected that the isoelectric point of histidin is considerably lower than that of either arginin or lysin. A further advantage possessed by this method lies in the fact that the coloring matter contained in the protein hydrolysate migrates to the anode and a clear colorless cathode solution is obtained.

The cathode solution is freed from sulphates by the addition of $Ba(OH)_2$ in slight excess and the excess of barium is removed by means of $CO_2$. From the filtrate, containing arginin and lysin, the former can be quantitatively separated by the addition of a concentrated alcoholic solution of picrolonic acid in an amount equivalent to the arginin present. The filtrate, after removal of the arginin picrolonate, is freed from a trace of picrolonic acid by extraction with ether in the usual manner. Lysin can now be isolated from the concentrated solution by addition of picric acid.

Further work on the application of this method to the separation of amino acids is in progress.

DISTRIBUTION OF NITROGEN IN THE GELATIN HYDROLYSATE
BEFORE AND AFTER ELECTROLYSIS.

| | Nitrogen in original Hydrolysate.[1] | | Distribution of Basic Nitrogen in Gelatin as Found by Van Slyke,[2] %. | Cathode Solution after Electrolysis at $P_H$ 7.5.[3] | | Cathode Solution after Electrolysis at $P_H$ 5.5[4] | |
|---|---|---|---|---|---|---|---|
| | Nitrogen, Gms. | Distribution of Basic Nitrogen, %. | | Nitrogen, Gms. | Distribution of basic Nitrogen, %. | Nitrogen, Gms. | Distribution of Basic Nitrogen, %. |
| Total nitrogen | 61.0 | | | 6.21 | | 0.99 | |
| Total basic nitrogen | 18.5 | | | 6.19 | | 0.99 | |
| Arginin nitrogen | 10.1 | 55 | 58 | 4.70 | 76 | 0.62 | 63 |
| Histidin nitrogen | 3.6 | 19 | 17 | 0 | 0 | 0.15 | 15 |
| Lysin nitrogen | 4.8 | 26 | 25 | 1.49 | 24 | 0.22 | 22 |

## ABSTRACTS OF THE COMMUNICATIONS, MINNESOTA BRANCH.

### Fourth meeting.

*Minneapolis, Minnesota, April 12, 1922.*

168 (1915)

### Immunologic studies of actinomycetes, with special reference to the acid-fast species.

By **EDMOND NELSON** and **ARTHUR T. HENRICI.**

[*From the Department of Bacteriology and Immunology, the University of Minnesota, Minneapolis, Minn.*]

The serums of six out of eight actinomycotic cattle fixed complement with four different antigens prepared from *Actinomycetes* isolated aërobically from lesions in cattle, and not with antigens prepared from saprophytic strains. The complement fixation test may be of diagnostic value in this disease.

The serum of a rabbit immunized against *A. bovis* fixed complement with antigens prepared from *A. bovis* and *A. maduræ*, but not with antigens prepared from the acid-fast varieties *A. asteroides* and *A. gypsoides*, or from *Mycobacterium tuberculosis*. The serums of rabbits immunized against *A. gypsoides* fixed complement equally with antigens prepared from *A. gypsoides* and *A. asteroides*, and in lower dilutions with *M. tuberculosis*, but not at all with *A. bovis* and *A. maduræ.* The serums of rabbits immunized against *M. tuberculosis* fixed complement with the homologous antigen, and in lower dilutions, with antigens prepared from the acid-fast *Actinomycetes*, *A. asteroides* and *A. gypsoides*, but not with *A. bovis* and *A. maduræ.* It would seem, therefore, that the acid-fast *Actinomycetes* are more closely related to the acid-fast bacteria than to the non acid-fast Actinomycetes.

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[1] 500 gms. of gelatin were hydrolyzed and the volume was brought to 2,000 c.c.

[2] Van Slyke, D.D., *J. Biol. Chem.*, 1911, x, 49.

[3] 1,000 c.c. of the original hydrolysate were used for this experiment.

[4] 130 c.c. of the original hydrolysate were used in this experiment.

*A. gypsoides* does not secrete a soluble toxine, but forms a very protent endotoxine. By careful vaccination there can be produced in rabbits an active protective immunity. The serum of such rabbits injected into guinea pigs gives the latter partial protection.

### 169 (1916)

## The effect of sodium benzoate and sodium hippurate, and other drugs upon the glomerular circulation in the frog.

By **RAYMOND N. BIETER** and **ARTHUR D. HIRSCHFELDER.**

[*From the Department of Pharmacology, University of Minnesota Minneapolis, Minnesota.*]

The effect of injection of sodium benzoate and sodium hippurate on the circulation through the glomeruli of frogs' kidneys was studied by Richards's[1] method of direct observation. Sodium benzoate, .008 to .020 mil of a ten per cent. solution per gram frog, invariably causes increased circulation through the glomeruli, increasing both the number of functionating glomeruli, the number of active loops in each glomerulus, and the velocity of flow in each capillary. Sodium hippurate, .008 to .020 mil of a fourteen per cent. solution per gram frog, had the opposite effect. This harmonizes with the work of H. B. Lewis,[2] and F. B. Kingsbury and W. W. Swanson,[3] who demonstrated that sodium hippurate was excreted more slowly than sodium benzoate in the mammal.

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[1] Richards, A. N., *Am. J. Med. Sc.*, 1922, clxiii, No. 1.

[2] Lewis, H. B., *J. Biol. Chem.*, 1914, xviii, 225. Lewis and Karr, *J. Biol. Chem.* 1916, xxv, 13.

[3] Kingsbury, F. B., and Swanson, W. W., *Arch. Int. Med.*, 1921, xxviii, 220–36.

This phenomenenon is of particular interest because Bunge and Schmiedeberg[1] demonstrated that hippuric acid is synthesized from benzoic acid and glycocoll in the kidney, while hippuric acid merely passes through it. It is interesting to speculate upon the possibility that the process of synthesis may bring about or be associated with a local vasodilation.

Among other drugs studied, nitroglycerine, one five-thousand solution, theobromine, one per cent. solution, and sodium indigo sulphonate, one per cent. solution, increased the glomerular circulation in a similar manner; and indigo sulphonate definitely stained the glomerular capsule blue. This shows that sodium indigo sulphonate is excreted through the glomeruli as well as through the tubules. Heidenhain[2] had demonstrated the excretion of this substance through the tubules but not through the glomeruli.[3]

### 170 (1917)

## The bile factor in pancreatitis.

By **FRANK C. MANN** and **ALFRED S. GIORDANO**.

[*From the Division of Experimental Surgery and Pathology, University of Minnesota, Rochester, Minnesota.*]

We have investigated the bile factor in pancreatitis from two chief aspects, the anatomic and the experimental.

Anatomically, two mechanisms have been suggested whereby bile can be passed into the pancreatic duct. One is based on the possibility that an obstruction could occur at the exit of the common bile duct in a manner to convert the two ducts into a continuous channel. We studied the relationship of the common bile duct to the pancreatic duct and their mode of entrance into the duodenum in man in order to determine the percentage of instances in which there would be an anatomic basis for the foregoing hypothesis. Our data conclusively prove that the number of instances in which the anatomic arrangement in the relationship of the two ducts would permit bile to pass into the pancreatic duct is very small. The other possibility that the sphincter at the duodenal end of the common bile duct could contract and convert the two ducts into a continuous channel has been investigated. Our data show that in most instances in man the sphincter is located at a point where contraction will close both ducts and will not convert them into a continuous channel. In a very small percentage of instances a small bundle of muscle fibers is found in a position where possibly it could convert the two ducts into a continuous channel. While there is an anatomic basis for the possibility of converting the two ducts into a continuous channel, either by mechanical obstruction or possibly by the action of a sphincter muscle, the percentage of instances in which this could occur is very small.

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[1] Bunge, G. and Schmiedeberg, O., *Arch. exper. Path. u. Pharmacol.*, 1876, vi, 233.

[2] Heidenhain, *Pflüger's Arch. f. d. ges. Physiol.*, 1874, ix, 1; Hermann's Handbuch der Physiol., 1883, v, 279–373.

[3] We desire to thank Dr. F. B. Kingsbury for furnishing the sodium hippurate and for suggesting this phase of the problem.

Experimentally we followed three lines of investigation: (1) to estimate the possible pressure the existing physiologic mechanism could exert to inject bile into the pancreatic duct; this pressure we have found to be relatively low; (2) to inject sterile bile into the pancreatic duct at the maximal pressure that could occur in the common bile duct; this did not produce typical hemorrhagic pancreatitis, although definite damage of the pancreas sometimes occurred; and (3) to ligate the common bile duct in goats (a species in which the main pancreatic duct opens into the common bile duct); this does not produce acute pancreatitis.

Our investigation has proved that an anatomic and physiologic basis for the theory that reflux of bile may occur in the pancreatic duct does exist. The evidence indicates that such a reflux of bile may rarely be the cause of chronic pancreatitis. The number of instances in which the necessary anatomic conditions are present for such an occurrence is very small. The possibility of bringing into play a physiologic mechanism which can infiltrate the pancreas with sterile bile to an extent actually to produce acute pancreatitis is questionable. Granted that the necessary anatomic, physiologic and pathologic factors are present and that the reflux of sterile bile under such conditions does produce pancreatitis, such cause for the condition must be very rare; few cases are on record. A reflux of bile could not have been the cause in any of our cases of acute pancreatitis. It should be noted that any mechanism which will afford the possibility for bile to pass into the pancreatic duct will also obstruct the flow of pancreatic juice. Furthermore, bile has been found in the pancreatic duct without acute pancreatitis. Pathologists should, in all cases of pancreatitis, examine the relationship of the two ducts to the duodenum and to each other in order to determine if it is anatomically possible for bile to pass into the pancreatic duct. Our data conclusively prove that we must look elsewhere for the explanation of the cause of most cases of pancreatitis.

### 171 (1918)

## A rapid method for the determination of the moisture content of expressed plant-tissue fluids.

By **ROSS AIKEN GORTNER** and **WALTER F. HOFFMAN.**

[*From the Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

The moisture content of expressed plant saps can be measured by determining the refractive index of the sap using an Abbé refractometer provided with a special "sugar scale."

In a series of determinations we have found that more accurate results can be obtained by the refractometric method than can be obtained by drying weighed portions of the saps in a vacuum oven. The results are fully as accurate as are those obtained by drying in vacuo at room temperature over sulfuric acid.

The great advantage of the method lies in the fact that only 2 or 3 drops of sap are necessary and that the entire time of measurement need not exceed two minutes.

It appears probable that the method may be applied to other biological fluids. A more extended account of the method will appear in a botanical journal.

### 172 (1919)

## A method for the estimation of the hydrophilic colloid content of expressed plant-tissue fluids.

By **ROBERT NEWTON** and **ROSS AIKEN GORTNER.**

[*From the Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

The freezing-point depression of the freshly expressed plant juice is first obtained. Then, having determined the total solids by the refractometric method described by Gortner and Hoffman in the preceding note, a quantity of sucrose just sufficient to make a molar solution in the total water present is added. The freezing-point depression is again determined, and is usually found to have increased more than the theoretical amount (2.085°, allowing for the formation of sucrose hexahydrate).

It is assumed that the magnitude of the excess depression is a measure of the quantity of water held in such a way as to be unavailable for the solution of the sugar. This has been found to correspond in a general way with the content of hydrophilic colloids, as indicated by viscosity measurements, and proved by dialysis of the juice where this has been carried out, as well as by the preparation of colloidal solutions of known composition. Preliminary experiments with gum arabic indicate a close relationship between the "bound" water and the concentration of the added colloid.

It seems probable that the method may be applied to any biological fluid. A more detailed account of the experiments will be published in a botanical journal.

### 173 (1920)

## Calcium phosphate metabolism showing the prevention of rickets by feeding clear grades of flour.

By **J. F. McCLENDON.**

[*From the Laboratory of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.*]

In the milling of flour the ideal seems to have been production of the whitest possible patent flour for human consumption, and the clear grades of flour, although they contain no shorts or bran, are too yellow in color for the American market. They contain much more phosphate than patent flour. The grain itself varies in phosphate content. Lack of available phosphate in the soil, or certain climatic conditions, may cause a reduction of phosphate content of grain. D. C. Mebane and myself showed that on a diet containing 45 per cent. of low phosphate rye (grown on peat soil) rickets was produced in white rats, whereas if this was substituted by high phosphate rye (from peat land in which the soil phosphate was made available by burning) no rickets developed. Soft winter wheat from the Ohio valley may be low in phosphate, and patent flour made from this wheat may contain as low as 0.075 per cent. P. Such flour was used in making a diet for white rats and produced severe rickets, whereas patent flour from hard spring wheat produced milder rickets, and Graham flour, no rickets. The percentage of phosphate in the patent flour depends, however, on the process of milling. At the Minnesota State Flour Mill some patent flour was made from the third middlings and contained 0.072 per cent. P, whereas the second clear flour contained 0.297 per cent. P. This, together with Graham flour, which I made by grinding hard winter tempered wheat containing 3.55 per cent. P, was used in making the following diets: Diet 96—NaCl 2 per cent., plaster of paris 2 per cent., yeast 1 per cent., spinach 1 per cent., lactalbumin 10 per cent., cotton seed oil 20 per cent., low phosphate flour 54 per cent., $NaHCO_3$ 10 per cent. Diet 98 was the same except there was no $NaHCO_3$ and the flour was 64 per cent. Diet 99 was the same as diet 98 except the second clear flour was used. Diet 100 was the same except that Graham flour was used.

| | Diet. | | | |
|---|---|---|---|---|
| | 96 | 98 | 99 | 100 |
| P, per cent. | 0.120 | 0.133 | 0.287 | 0.340 |
| Ca, per cent. | 0.607 | 0.621 | 0.645 | 0.635 |

Four rats of litter 23, twenty days old, weighing 27 ± 1 gm. were taken and placed on these diets. From the 33d to the 40th day of age they were kept in metabolism cages and the calcium phosphate metabolism determined with the following results:

| Rat No. | Sex. | Diet. | Grams. | | | | Milligrams per Day. | | | | | | X-Ray 46 Days Old. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Body Weight. | | | Food Intake per Day. | P. | | | Ca. | | | |
| | | | 33 Days. Old. | 40 Days Old. | Gain | | In. | Out. | Ret. | In. | Out. | Ret. | |
| I | o | 96 | 33 | 34 | 1 | 3.49 | 4.2 | 3.1 | 1.1 | 21.2 | 19.7 | 1.5 | Rickets |
| II | ♀ | 98 | 36 | 42 | 6 | 3.82 | 5.1 | 3.5 | 1.6 | 23.7 | 22.7 | 1.0 | Rickets |
| III | ♀ | 99 | 47 | 65 | 18 | 7.50 | 21.4 | 13.8 | 7.6 | 48.4 | 38.1 | 10.3 | No Rickets |
| IV | o | 100 | 52 | 73 | 21 | 7.36 | 25.0 | 15.8 | 9.2 | 46.7 | 32.7 | 14.0 | No Rickets |

It will be seen from the table that diets 99 and 100 containing the second clear and the Graham flours caused retention of P and Ca and prevented rickets, other things being equal. The X-ray plate suggested a very mild rickets in the rat eating Graham flour. Diet 96, containing high alkali, stopped the growth as well as produced rickets. We may conclude that it would be safer to feed infants Graham bread instead of white bread, but if the Graham bread is too laxative, bread may be made of second clear flour, which is not laxative, and rickets be prevented with more certainty than with the Graham flour.

*Methods.*—The rats were placed in wire cages sitting in six-inch silica dishes and at the end of the metabolism period the cages were lifted out, the dishes placed in a muffle and ashed at the lowest possible temperature in an atmosphere of $O_2$. The ash was then dissolved in dilute nitric acid and evaporated and redissolved and boiled.

*Ca Analysis.*—Take aliquot containing 10–50 mg. Ca in a 200 c.c. pyrex flask, add 1 drop brom-phenol blue, and 20 c.c. 2½ per cent. oxalic acid; add 20 per cent. $Na_2CO_3$ drop by drop until color changes to lavender; boil; stopper and shake one hour; filter; wash; transfer precipitate back to 200 c.c. flask with 100 c.c. $H_2O$; add 5 c.c. conc. $H_2SO_4$; heat to 75° and titrate drop by drop with 0.1 N

$KMnO_4$ (1 c.c. = 2 mg. Ca); add the filter paper to flask and titrate to find end point. Tenth normal oxalic acid (made from powdered oxalic acid dried over a mixture of hydrated and dehydrated oxalic acid) is used to standize the permanganate (as well as the NaOH for P analysis).

*P Analysis.*—Take aliquot containing 5–10 mg. P in a 200 c.c. pyrex flask; dilute to 100 c.c; add 10 c.c. $HNO_3$ and 1 drop brom-phenol blue; neutralize with ammonia; add 20 c.c. acid ammonium molybdate solution (usual formula); heat to 65°; shake 5 minutes; filter (the filtrate should turn methyl violet green). Wash until there is no titratable acidity in wash water; transfer paper and precipitate back to flask; run in 0.1 N NaOH until precipitate dissolves; boil 5 minutes; add ½ c.c. phenolphthalein and titrate to colorless with 0.1 N HCl. 1 c.c. alkali corresponds to 0.1194 mg. P.

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### 174 (1921)

## The agglutination reaction in the diagnosis of tuberculosis.

By **W. P. LARSON, E. N. NELSON** and **PU YUNG CHANG**.

[*From the Department of Bacteriology and Immunology, University of Minnesota, Minneapolis, Minn.*]

Many attempts have been made in the past to make use of the agglutination reaction in the diagnosis of tuberculosis. The test has been found unsatisfactory largely because of the fact that the tubercle bacilli grow in adherent masses from which it has been difficult to prepare the homogeneous suspensions necessary for carrying out the test.

In the year 1918 Larson, Hartzell and Diehl[1] described a method of emulsifying and disrupting bacteria by subjecting them to the influence of carbon dioxide under high pressure, after which the pressure was suddenly released, causing a disruption of the organisms as a result of the rapid escape of the gas with which they were filled.

Tubercle bacilli grown on glycerine broth or glycerine agar are suspended in distilled water and placed in the apparatus where they are subjected to $CO_2$ pressure for two or more hours. The process may be repeated as often as desired although one treatment, as a rule, is sufficient to effect emulsification. By repeating the process several times a large percentage of the organisms may be disrupted. After the $CO_2$ treatment the emulsion is diluted to the desired standard with salt solution. In this way a perfectly homogeneous suspension of tubercle bacilli, which gives no sediment after standing several days without agitation, may be obtained. The addition of 0.2 per cent. trikresol enables the suspension to be kept in the laboratory indefinitely.

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[1] *Jour. Inf. Diseases*, 1918, xxii, 271–279.

We have performed the agglutination reaction on three hundred cases, one hundred of which were known to have tuberculosis, and two hundred "normal" cases. With one exception all of the tubercular cases gave agglutination. Of the "normals" all but five gave a negative reaction. Of the cases five which gave the positive reaction four were suspected of having syphilis but only one of these had ever given a positive Wassermann. The tests were carried out by the macroscopic method. The serums were diluted 20, 40, 80, 160, 320 and 640 times respectively, placed in the incubator for two hours, and in the ice-box over night.

In this series of dilutions a proagglutinoid zone was noted in a majority of the positive cases, in that agglutination was rarely present in the tube containing serum in dilution of 1–20. Agglutination was very marked, often complete precipitation, in dilutions 40, 80, and 160 and somewhat irregular in the higher dilutions. It is important that the serums used be free from hemoglobin since we have found that hemoglobin causes a false agglutination of the tubercle bacillus. From these results we believe the agglutination test will prove of value in the diagnosis of tuberculosis.

### 175 (1922)

## Correlations among the constituents of potato tubers.

By J. J. WILLAMAN and R. M. WEST.

[*From the Minnesota Agricultural Experiment Station, St. Paul, Minn.*]

It has long been known that both the total dry matter and the starch content of potato tubers are proportional to the specific gravity; in fact in factory practice Märker's[1] table of specific gravities is commonly used in assaying potatoes for starch. Also, the starch content and dry matter are correlated positively with mealiness, whereas high protein causes sogginess. According to American standards mealiness is desirable; in Europe the reverse is true. Therefore it would seem that attempts to develop a high protein potato and still to maintain desirable culinary properties were doomed to failure, although East[2] was convinced otherwise, provided a high dry-matter content be maintained.

In 1911 56 samples of potatoes, representing 4 varietal groups, and grown under various conditions in different parts of Minnesota, were collected and analyzed at this station, with a view to determining the factors which affect the composition. It seemed desirable to utilize these data still further by calculating the coefficients of correlation among all the constituents of the tubers. The results of these calculations appear in Table I. Both starch and soluble sugars were determined, but the sum of the two only is here presented. The starch represents about 95 per cent. of the two.

It will be seen that there is an intimate relation between specific gravity and dry matter, which is to be expected. It will be further noticed that there is no correlation whatsoever between carbohydrate and dry matter and between nitrogen and dry matter. This is not contrary to the relation stated above between specific gravity and starch, since in factory practice the starch content is considered on the wet basis, and in this table all calculations are on the dry basis. The nitrogen and carbohydrate vary inversely with each other, which is naturally the case with the two main constituents of a tissue, totaling 80 per cent. of the dry matter. Also as the dry matter increases the ether extract increases, and the ash decreases.

These relations can be stated in another way: A higher dry-matter content does not simply mean a loss of moisture, else there would be no change in the ratios of constituents. The formation of a higher dry matter content means the laying down of more ether extract material, as evidenced by the coefficient of + .333

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[1] Märker, M. H., *Landw. vers. Stat.*, 1880, xxv, 107.

[2] East, E. M., Ill. Agr. Exp. Sta., 1908, Bull. 127, p. 135.

± .078. It must also mean the simultaneous laying down of more protein and carbohydrate, for the following reasons: (1) the amount of ether extract found in tubers is insufficient to account for all the increase in dry matter; (2) the protein and carbohydrate are sufficient to account for it; (3) the protein and carbohydrate are not correlated with the dry matter, but are correlated with each other negatively. As these organic constituents are increased the minerals are decreased, as is evidenced by the coefficient of −.380 ± .076 between the ash and dry matter. In fact the ash bears a negative relation to all the other factors studied, although in the majority of cases the coefficient is too small to be of significance.

From the above data we can conclude that it should be entirely possible to improve the potato tuber as regards protein, provided the dry matter be increased. Mealiness can then still be maintained. This improved tuber will probably be spheroidal, instead of long in shape, since the longitudinal diameter is correlated positively with the starch content.[1]

TABLE I. SUMMARY OF CORRELATIONS AMONG THE VARIOUS FACTORS IN POTATO TUBERS.

| | Dry Matter. | Nitrogen. | Ash. | Ether Extract. | Starch plus Sugar. |
|---|---|---|---|---|---|
| Specific gravity | +.637±.054 | −.233±.084 | −.311±.082 | −.164±.087 | +.218±.086 |
| Dry matter.... | | +.034±.090 | −.380±.076 | +.333±.078 | +.059±.090 |
| Nitrogen...... | | | −.052±.090 | +.216±.086 | −.590±.058 |
| Ash.......... | | | | −.116±.088 | −.223±.085 |
| Ether extract.. | | | | | +.004±.090 |

### 176 (1923)

## Yeast as a source of vitamine-B for the growth of rats.

By **CORNELIA KENNEDY** and **LEROY S. PALMER.**

[*From the Section of Animal Nutrition, Division of Agricultural Biochemistry, University of Minnesota, St. Paul, Minn.*]

Yeast is commonly considered the richest source of vitamine-B for the growth of young animals. We have fed groups of rats in colonies on a basal diet of purified casein 18 per cent., salts 3.7 per cent., agar 2 per cent., butter fat 5 per cent., with dextrin to make 100 per cent., and have supplied the vitamine-B in the form of dried yeast of various sources, both as an integral part of the diet or separately in the form of a tablet. Our results were as follows:

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[1] Renski, M. D., Abs. in *Exp. Sta. Rec.*, 1911, xxiv, 439.

Air-dried Fleischmann's baker's yeast containing 40 per cent. yeast in the dried product failed to produce normal growth in all cases when the ration contained 4 per cent. or less of the dried product. and certain individuals even failed to make normal growth when the dried yeast formed 10 per cent. of the diet. Out of 20 rats, 9 were females, and none of these produced young during the 2 to 4 months of the experiment.

When the same yeast was fed separately, 0.6 gram per day per rat was required to secure normal growth. Two out of 4 females on this diet produced young (total of 3 litters) but all the young were destroyed by the mothers.

A dried brewer's yeast prepared by us from a wet mixture of bottom yeast and wort secured from a local brewery produced only about one-half normal growth when fed as high as 10 per cent. of the diet, but when these rats were transferred to a mixed diet of grains and milk their growth curves rose sharply towards the normal. When the same yeast was fed separately to other rats at the rate of 0.2 and 0.4 gram per day per rat the results were little better than when the yeast was incorporated in the diet at the rate of 10 per cent. There was no reproduction.

*Saccharomyces cerevisiæ* was grown by us in a wort of malt extract which also contained a little extract of hops. The filtered, washed yeast was air-dried. When fed at the rate of 0.2 gram a day as a supplement to the basal vitamine-B-free ration the rats made a continuous slow growth but the mature rats were undersized, the males averaging about 200 grams and the females about 160 grams. One female had 2 litters, but the young all died in the first case and in the second case they were so poorly nourished that they had not left the nest after 4 weeks. These young rats later died in convulsoins after they had become large enough to eat their mother's ration.

Young rats fed a dried distiller's yeast at the rate of 0.2 gram daily made poor growth or none at all during a period of 4 weeks.

Fleischmann's starch-free yeast air-dried by us did not produce normal growth when 0.2 gram of the dried yeast was fed daily. Growth was not stimulated appreciably even when the amount was increased to 0.6 gram daily although one female produced a litter of 3, two of which died soon after birth and the remaining one of which never grew normally. Better results were secured for a time in the case of other rats fed 0.2 gram daily of dried starch-free yeast furnished us by the Fleischmann Yeast Co. in the dried form but this growth was not maintained when the yeast was increased to 0.4 gram daily. At the end of 16 weeks all these rats were undersized and no young were produced.

Our results show clearly that yeast is a variable source of vitamine-B for growth and cannot be accepted as a standard product in experiments in which a vitamine-B preparation is required. Its efficacy depends upon the manner in which it is fed, the species of yeast, and apparently also on the character of the wort in which it is grown. Our results do not support the general belief that yeast is an unusually rich source of vitamine-B for growth.

Especially striking were the consistent failures of the rats fed yeast as the sole source of vitamine-B to reproduce normally. In fact, in most cases no reproduction was secured, although each colony contained animals of both sexes. These results, especially, have raised grave doubts in our minds as to the suitability of yeast as a source of vitamine-B in nutrition experiments.

# SCIENTIFIC PROCEEDINGS.

## Abstracts of Communications.

### One hundred twenty-fourth meeting.

*Columbia University, May 17, 1922.*
*Vice-President Jobling in the chair.*

177 (1924)

### The Hecht-Weinberg-Gradwohl reaction in syphilis.

By **L. W. FAMULENER** and **JULIA A. W. HEWITT.**

[*From the Pathological Laboratory, St. Luke's Hospital, New York City.*]

A comparative study was made of the results obtained by the Hecht-Weinberg-Gradwohl and a modified Wassermann technique in the serum diagnosis of syphilis during the routine examination of a general group of hospital patients. An acetone-insoluble antigen, as advised by Gradwohl, was used in both methods, and in addition, a cholesterinized antigen with the Wassermann method. In performing the Hecht-Weinberg-Gradwohl test, the procedure as outlined by Gradwohl was closely followed. The Wassermann technique was that which had given satisfactory results in St. Luke's Hospital during the past few years. Two series of tests were undertaken; the first series included 50 cases, the second 100 cases. In each instance the "hemolytic index" as defined by Gradwohl, was carefully determined, and his test was performed only on those sera which showed an index of 4 or greater, since he states that the test possesses no advantage over the Wassermann method when the index is 3 or lower. The first series showed only seven (14 per cent.) sera which filled that requirement. Since the erythrocytes used in this series were from an old laboratory sheep, it was thought that possibly its blood cells had become more resistant to hemolysis than normally, so a second animal, which had been bled but little previously, supplied the erythrocytes for the second series of 100 cases. In this series, 34 per cent. of the sera showed the natural hemolytic index desired. All sera were tested either shortly after the blood was drawn, or within 24 hours; the latter were kept in the icebox until used.

Of the seven suitable sera in the first series, only one gave a positive reaction with both methods. Another gave a questionable positive reaction with the Hecht-Weinberg-Gradwohl method, while with the Wassermann method, a negative resulted with the acetone-insoluble antigen, but a low positive with the cholesterinized antigen. The remaining five sera gave negative reactions by both methods. In the second series of tests, 34 were found of sufficiently high hemolytic value to be used advantageously in comparative tests. Of these, 28 gave negative results with both methods. Five sera gave positive reactions with the Hecht-Weinberg-Gradwohl technique; four of which gave similar reactions by the Wassermann method, and one was negative. The latter was a serum from a treated luetic patient with which the Hecht-Weinberg-Gradwohl test gave only a low positive (+) reaction; however, the Wassermann method gave a doubtful (±) reaction using the cholesterinized antigen with this serum. The Wassermann method also gave a strongly positive (++++) with the cholesterinized antigen in one case where the acetone-insoluble antigen gave a negative result, as well as the Hecht-Weinberg-Gradwohl test. This patient gave a history of a syphilitic infection ten years previously, and had undergone early treatment. All sera in the above series which gave positive reaction in any of these tests came from patients whose history supported the findings, except in one case, where it was quite suspicious.

While our series of tests are too small for definite conclusions, they suggest that possibly under certain circumstances, the Hecht-Weinberg-Gradwohl test might be superior to the Wassermann method when an acetone-insoluble antigen is used. But on the other hand the latter method with a cholesterinized antigen probably gives better results with known infected cases.

### 178 (1925)

## Pneumococcus grouping on a thousand cases.

By **L. W. FAMULENER** and **LUCILE ROBEY**.

[*From the Pathological Laboratory, St. Luke's Hospital, New York City.*]

An analysis of the results obtained by the pneumococcus grouping test (Rockefeller Institute method) performed upon 1,000 sputa from hospital patients suffering from acute respiratory infections showed, roughly, that only 80 per cent. of the specimens submitted for examination yielded a sufficient number of pneumococci for group determination. The washed sputum was either injected intraperitoneally into a mouse, or cultured in the Avery medium, or by both methods in some instances, for a growth of the organism. The presence of the pneumococcus was determined by its morphological characteristics in stained preparations, and by its bile solubility. The remaining 20 per cent. of sputa either failed to show the pneumococcus in culture, or it was present in such scant numbers that the serological test was considered to be of a negative character. In general, those sputa yielding negative results came from patients suffering from influenza, bronchopneumonia, pulmonary tuberculosis, etc. Stained preparations of materials or cultures from such cases usually revealed either the streptococcus, staphylococcus, *B. influenzæ*, or *B. Friedlander* (very rarely), as the predominating organism. Not infrequently the specimen submitted to the laboratory consisted principally of saliva, so it could not be considered a true sputum, as only the usual mouth organisms developed in culture. Therefore the latter group of tests will be excluded from further consideration in this report, and attention will be directed to the group where the laboratory tests gave positive results.

The study embraces approximately 800 cases which were admitted to St. Luke's Hospital during the years 1918 to 1921, inclusive. The average percentage incidence of each group of pneumococci for this period was as follows: Group I, 11.5 per cent.; Group II, 4.9 per cent.; Group III, 13.6 per cent.; and

Group IV, 70 per cent. The highest incidence for all groups was during the months of January, February, and March, with a decided drop in the late spring and summer months. It is interesting to note that during these four years, no Group II pneumococcus cases, in a series of 40, occurred in the months of June, July, and August. But 12.9 per cent. of the cases (93) belonging to Group I, 8.2 per cent. of cases (110) belonging to Group III, and 8.7 per cent. of the cases (570) belonging to Group IV, occurred during these summer months.

Further analyses of the data mentioned in the foregoing preliminary report are in preparation, the results of which we hope to report more fully later.

### 179 (1926)

## The hemolytic properties of the pneumococcus.

By **JULIA A. W. HEWITT** and **L. W. FAMULENER.**

[*From the Pathological Laboratory, St. Luke's Hospital, New York City.*]

Recently an interesting phenomenon was observed in culture plates made with the blood from a fatal case of septicemia with meningitis, which followed mastoiditis. The blood culture after 24 hours' incubation showed a considerable number of characteristic green colonies which proved to be pneumococcus, Group IV. One of the culture plates which had been used for demonstration purposes before a class of students was stored in the ice box to be preserved for a later section. Some days later, upon its removal from the refrigerator, it was found that marked zones, simulating hemolysis, had appeared about the colonies, giving an appearance almost identical to that produced by hemolyzing types of streptococci. In our previous experience with pneumococcus blood-culture plates, no hemolyzing effect of this nature had been noted, although no continued observation under similar conditions had been followed. The standard reference- and text-books on bacteriology consulted failed, with one exception, to note that pneumococcus colonies might produce hemolysis in blood-agar plates. Zinsser[1] states that hemolysin production, which occurs late, is slight but definite with the pneumococcus on blood plates. Brown[2] in his study upon the hemolyzing properties of the streptococcus, ascribes a hemolyzing action to the pneumococcus similar to that shown by his alpha type of streptococcus. As evident, considerable lack of agreement exists among different authorities concerning hemolysin production by the pneumococcus on blood-culture plates. The question remains open for further investigation.

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[1] Hiss-Zinsser-Russell, "Textbook of Bacteriology," 1922, 5th Ed., p. 445.

Fortunately, fishings of colonies from this particular strain had been made and cultured upon blood-agar slants. In order to determine if hemolysis is a common property of the pneumococcus, under certain circumstances, tests were performed under varying conditions with this, and other immunological types of the pneumococcus isolated at that time. A series of preliminary tests were first carried out as a guide in studying the problem. The results of these tests showed that under certain conditions the pneumococcus colony produced a hemolysis of the cells in the immediately surrounding blood-agar medium. The principal tests undertaken concerned in particular the question of medium reaction and the influence of temperature upon possible hemolysin production. The medium was prepared from a meat-infusion broth (500 gm. to a liter), to which was added 1 per cent. peptone (Fairchild's), and 2 per cent. shredded agar. The reaction of one portion was adjusted to $P_H$ 7.0 to 7.1, and the other to $P_H$ 8.0 to 8.1. It was tubed in 6 c.c. and 12. c.c. amounts and sterilized in the autoclave at 15 pounds' pressure for 30 minutes. To the agar medium at the time of plating the organism, sufficient freshly drawn, defibrinated, human blood was added to produce a 5 per cent. concentration in Petri dishes (9 cm. diameter), and the whole evenly distributed. By using different amounts of the medium, or slanting the dish, the poured inoculated medium gave layers varying from 0.5 mm. to 3.0 mm. in depth. Pneumococci representing all groups were grown on slanted 5 per cent. human blood beef-extract agar, and used for plating purposes after two to four days' incubation. A small amount of growth was removed and evenly suspended in plain broth. Inoculations were made directly from this into the special agar medium, poured, and mixed with the required amount of blood in the Petri dish. Identical platings were made from the same suspension of each organism in the study of the influence of medium reaction ($P_H$ 7.0 and $P_H$ 8.0) upon hemolysin production. For the icebox test, duplicate series of plates, each consisting of both the special neutral and alkaline media, were inoculated in parallel from the same bacterial suspensions, and incubated at 37° C. for approximately 36 hours. Then one series was removed and placed in the icebox at 8° C. for three days. The plates were carefully examined daily, and the results fully recorded.

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[2] Monographs of the Rockefeller Institute for Medical Research, 1919, No. 9 p. 23.

Only a brief summary of the results of this work can be considered, as the experimental data are too extensive to be recorded in this place. Important factors influencing the clearing or the degree of hemolysis about the colonies were found to be the thickness of the medium layer, and the relative position of the colony to the layer, *i.e.*, on the surface, embedded in layer, or sublayer (between medium and bottom of dish). Surface and sublayer colonies usually were large, but if the medium was rather thick (2 to 3 mm.), they ordinarily showed no surrounding hemolyzed zone. Colonies embedded in the thick layer rarely showed any clearing, but generally, after 36 to 48 hours' incubation, a coloration varying from a deep green, brownish green to almost black. In the thinner layers, they appeared pale green to grayish in color. If a clearing actually occurred immediately in contact with the deeply embedded colony, the outer deep green or brownish green zone of methemogoblin would fully envelope and mask the reaction. Colonies in the thinner layer which showed an immediate inner zone of clearing frequently were surrounded by an outer zone of coloration varying from a pale to a dark, or even brownish green. In general, the colonies which best showed the hemolyzing action were embedded in a blood-agar layer of one mm. or slightly more, and as growth advanced, tended to cause a slight uplifting of the medium. The hemolyzing action, when occurring, usually appeared after 48 hours' incubation, and reached its maximum extent in from 72 to 96 hours. The hemolyzed zone varied from a barely visible clear surrounding ring to one which was 1 or 2 mm. in diameter (occasionally greater), rarely very broad, such as is seen with the hemolyzing streptococcus.

Likewise it very exceptionally produced a similar degree of transparency in surrounding medium. Usually the zone about the pneumococcus colony is translucent, hazy, or of ground glass appearance, and frequently with an outer, more or less diffuse methemoglobin ring, varying in color from pale to brownish green. When examined under the low power of the microscope, the zone of clearing rarely was free from "shadow cells," and in those showing haziness, such cells were much more in evidence. If the zone appeared slightly opaque or pigmented, the blood cells showed certain amounts of the changed hemoglobin or methemoglobin present. The zone of clearing about the pneumococcus colony can hardly be considered a hemolysis in the sense as applied to that produced by the *Streptococcus hemolyticus*. The first is probably due to an intracellular hematoxin, liberated by autolysis of organisms in the colony, while the second is probably extracellular, elaborated and passed out by the living organism. In the case of the pneumococcus, perhaps it would be more appropriate to designate the change as a pseudo-hemolysis, since it lacks the completeness of action shown by the hemolytic streptococcus. Further, the reaction is probably complicated by two independent processes occurring at the same time—methemoglobin formation induced by the living organisms, and a hemolysis produced by a hematoxin arising from autolyzed cells, as suggested by Cole's studies on pneumococcus hematoxin,[1] and methemoglobin production by the pneumococcus.[2]

The hemolyzing action of different pneumococcus strains within the same group was found rather irregular; certain ones appeared to possess that ability to a greater degree than others. Even the same strain on repeated tests showed considerable variation.

The medium reaction ($P_H$ 7.0 and $P_H$ 8.0) in which the organisms were plated produced no apparent variation in growth or hemolytic action at incubator temperature. Also no appreciable difference in hemolysis could be recognized in a similar series of plate cultures, which were placed in the icebox three days after a primary incubation of 36 hours. Methemoglobin production was inhibited in the icebox, but progressed in the controls which were left in the incubator. No inhibition to hemolysis, as occurs with the *Streptococcus hemolyticus*, was observed with the pneumococcus plated in a medium ($P_H$ 7.8) containing 1 per cent. dextrose. These plates were first incubated three days, then stored in the icebox three days.

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[1] *Jour. Exper. Med.*, 1914, xx, 346.

[2] *Jour. Exper. Med.*, 1914, xx, 363.

Fishings of pneumococcus colonies which showed markedly clear zones in blood plates were cultured on blood-agar slants. When replated these cultures produced colonies which failed to show any pronounced differences from the original cultures.

The hemolysis probably depends, among other factors, on depth of agar layer, the percentage of blood corpuscles present, and the age of the colony, and its vitality. A number of other possible factors might enter this reaction which cannot be discussed in this paper, but we hope that others may take up this problem more fully and investigate the question.

## CONCLUSIONS.

In conclusion, our results would indicate that, (*a*) pneumococci of all serological groups, under certain cultural conditions, may hemolyze human erythrocytes, and, (*b*) apparently, this property is not influenced by the reaction of the medium within the growth limits of the organisms, nor (*c*) by prolonged refrigeration of the developed colonies on blood-agar plates. (*d*) Probably the hemolysin is an intracellular product liberated from autolyzed organisms which diffuse from the colony into the surrounding blood agar.

180 (1927)

## Studies on the therapeutic effect of B. acidophilus milk and lactose.

By **NICHOLAS KOPELOFF** and **C. O. CHENEY**.

[*Bacteriology and Clinical Departments, New York State Psychiatric Institute, Ward's Island, New York City* ]

In a series of psychotic and normal (mentally) subjects relief from chronic constipation and diarrhea was obtained by the ingestion of *B. acidophilus* milk and lactose. This corroborates the work of Rettger and Cheplin.

The normal subjects as well as the psychotic patients receiving treatment, gained in weight; but while the latter were improved physically, there was no improvement in their psychoses.

The intestinal flora becomes transformed on treatment with *B. acidophilus* whole milk and lactose, but the relative percentage of gram-positive rods rarely exceeds 70 per cent.

Incubating *B. acidophilus* whole milk at room temperature is satisfactory for only a few days, after which the number of viable organisms decrease rather rapidly and the acidity increases to the point of unpalatability.

### 181 (1928)

## Observations on the behavior of the nucleus and chromosomes in spermatocytes of Lasiopogon (Diptera).

By CHAS. W. METZ and JOSÉ F. NONIDEZ.

[*From the Carnegie Institution of Washington, Cold Spring Harbor, N. Y., and Cornell University Medical College, New York City.*]

In the primary spermatocytes of *Lasiopogon* (species not yet determined) the earliest growth stages resemble those in other asilids (*e.g., Asilus sericeus*);[1] but before growth has progressed very far, the nucleus, which previously has been approximately spherical, becomes irregularly invaginated and evaginated. The nuclear membrane appears to push in around the chromosome threads and the latter to push out into the cytoplasm; so that soon each thread (bivalent) lies in a lobe or pocket, isolated to a great extent from the others. The nucleus becomes converted almost entirely into lobes, which follow the contour of the chromosomes and ramify in various directions through the cytoplasm. There is no uniform configuration; the chromosome threads are long and slender, and often follow a tortuous path, apparently at random save for a slight polarization toward the nucleolus.

As growth progresses the chromosome threads condense and shorten, and coincidently the lobes become less ramifying. This process continues up to the metaphase of the first division, when the chromosomes are fully condensed. The nuclear outline is still persistent at this time and still exhibits an irregular contour, conforming to that of the five bivalent chromosomes.

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[1] Metz and Nonidez, *Jour. Exp. Zoöl.*, 1921, xxxii, 165.

Aside from the unusual nature of these processes, which, so far as we know, have not hitherto been found in maturation stages, two features appear to be of interest. First is the fact that as the nuclear membrane (or the cytoplasm) pushes in around the chromatin threads, it comes close to, but not in contact with, the threads. This is easily seen, for the nucleoplasm is transparent and the cytoplasm is dark, leaving the boundary, or nuclear membrane, clear cut in outline. If the lobe is cylindrical, as is frequently the case, the chromatin thread extends uniformly through the center (or near the center), like a slender core. This condition, in which the chromosome is separated from the nuclear membrane by a hyaline area of fairly constant thickness, is so uniform and persistent (obtaining throughout most of the growth period) that it can hardly be considered accidental. It suggests that around the bivalent chromatin thread there may be a cortical layer of dense or gelatinous material which holds off the nuclear membrane. This layer, whatever its nature may be, is usually from one to three times the thickness of the chromatin thread, and varies with the latter through the growth period, becoming thicker as the thread condenses.

That the nuclear membrane is held off through the instrumentality of the chromosome itself is indicated by the fact that the diameter of a lobe is usually greatest where the chromosome lies, and that where the lobe extends beyond the chromosome very far it becomes narrowed or closed.

The second point of interest has to do with the fact that the lobes of the nucleus, although often almost completely isolated from one another, apparently never become cut off as separate vesicles. Each seems to retain some connection, even if it is only a narrow channel, with one or more of the other lobes, so that the continuity of the nucleus is maintained.

182 (1929)

## Location of the earliest changes in experimental xerophthalmia of rats.[1]

By **A. M. YUDKIN** and **R. A. LAMBERT.**

[*From the Departments of Pathology and Physiological Chemistry, Yale University, New Haven, Conn.*]

It is generally assumed that the lesions in experimental xerophthalmia due to dietary deficiency have their origin in the cornea which, in the advanced stage of the condition, becomes markedly affected. A recent study of the eyes of a series of rats on a diet deficient in fat-soluble A has led us to the conclusion that it is the eyelid rather than the cornea that is primarily affected.

The following is a summary of the study. Six young rats, weighing from 45 to 50 grams, were placed on a diet consisting of casein, mineral salts, starch, lard, and yeast. After from 45 to 60 days, the first evidences of eye changes developed—watery lacrimation with a serosanguineous conjunctival secretion, becoming after a short time somewhat viscid. The rats were killed at this stage and the eyes, with the lids attached, were embedded and sectioned. In all cases early focal lesions were found in the epithelial lining of the lids. The changes consisted of localized foci of degeneration of the epidermis with cellular infiltration which, in some cases, extended into the subepidermal tissue. In all these early cases the cornea was found uninvolved, that is, there were no degenerative changes which could be recognized by ordinary stains, and no cellular or vascular reaction.

These findings suggest that the eye changes resulting from deficiency in fat-soluble A vitamine, which in the advanced stage is characterized by a widespread keratitis, do not begin in the cornea but have their origin in the lids. In this respect the sequence of events is the same as that in some of the severer types of acute and chronic conjunctivitis which are frequently complicated by corneal injury, with infection and ulceration of this structure.

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[1] This investigation was made in coöperation with Drs. Thomas B. Osborne and Lafayette B. Mendel, under whose direction the feeding of the experimental animals was conducted. The Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington shared the expenses of the research.

183 (1930)

## Lesions in the lacrimal glands of rats in experimental xerophthalmia.[1]

By **A. M. YUDKIN** and **R. A. LAMBERT.**

[*From the Departments of Pathology and Physiological Chemistry, Yale University, New Haven, Conn.*]

The marked changes in lacrimal secretion associated with experimental xerophthalmia, suggested a study of the lacrimal glands of animals suffering from this condition. Young rats weighing from 45 to 50 grams were subjected to a diet deficient in fat-soluble A. The diet consisted of casein, mineral salts, starch, lard, and yeast. There were 24 animals in the series including 6 with early eye changes, 8 with advanced changes, 4 cured cases and 6 normal controls. In the early 6 cases the lacrimal glands appeared little altered, although in some the glandular epithelium looked to be somewhat modified, the cells being markedly vacuolated and quite ragged in outline. Of the 8 advanced cases, one showed a widespread suppurative inflammatory process with polymorphonuclear leukocytes filling the tubules. In three there were foci of necrosis, which were quite numerous in one instance. In the four remaining cases the parenchymal cells were possibly slightly altered. Three out of four cured cases showed mononuclear cell accumulations, foci of atrophy, or fibrosis. In the glands of the six normal animals definite changes were found in one only.

From a review of all the material, we believe the following tentative conclusions are justified:

1. The lacrimal gland may be the seat of a marked pathological change, either degenerative or inflammatory in nature.

2. Such changes are much more marked in xerophthalmic than in normal rats.

3. Variations in the size, form, and staining properties of the cells are frequently seen and are probably referable to functional disturbances related to the ophthalmia.

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[1] This investigation was made in coöperation with Drs. Thomas B. Osborne and Lafayette B. Mendel, under whose direction the feeding of the experimental animals was conducted. The Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington shared the expenses of the research.

4. These changes may account for some of the phenomena of xerophthalmia, particularly the drying of the cornea in the later stage of the condition.

### 184 (1931)

## Increased blood sugar coincident with ovulation in pigeons.

By **HANNAH ELIZABETH HONEYWELL** and **OSCAR RIDDLE.**

[*From the Department of Physiology, Columbia University, New York City, and the Carnegie Station for Experimental Evolution, Cold Spring Harbor, N. Y.*]

The data of an earlier communication by one of us[1] have made it nearly certain that in healthy pigeons the suprarenals usually undergo extensive and regular enlargement at the period of ovulation. This result, in view of many facts which indicate an influence of the suprarenals on the mobilization of sugar, naturally leads to an inquiry as to whether the blood sugar also undergoes a similar and simultaneous increase in amount. The data reported here indicate that such an increase of blood sugar does also regularly occur.

Scott and Honeywell[2] concluded that in non-reproducing common pigeons of unknown sex the blood sugar amounts on the average to about 185 mgm. per 100 c.c. of blood as determined by MacLean's method. This same method was used in the present study and a similar amount of sugar was found for birds not actively ovulating. Ring doves in other than ovulation periods have, however, distinctly less blood sugar. Both of these kinds of pigeons, together with a third group—"scraggly" common pigeons—have been used by us. The "scragglies" are a mutational or aberrant form having a quite imperfect epidermal system (including the feathers) and bearing suprarenals earlier observed to show wide variation in size. Because of these variations it seemed desirable to include observations on this group. Males and females, both adult and young, of all the three groups have been studied, but only figures obtained for adult reproducing females are here considered.

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[1] Riddle, Oscar, PROC. SOC. EXPER. BIOL. AND MED., 1922, xix, 122.

[2] Scott, E. L. and Honeywell, H. E., *Amer. Jour. Physiol.*, 1921, lv, 362.

All samples were obtained by needle-puncture of the heart. Unfortunately, this procedure proved capable of producing occasional ovulations into the body cavity and also the resorption in the ovary of the nearly ripe ova. Since duplicate samples had to be taken from at least two different stages of ovulation it was possible to kill the bird only after the final sample was obtained. This circumstance and the disturbing effects of the puncture noted above unite to make it impossible to know in a few special cases at which stage with reference to ovulation the sample was taken. Such cases are indicated in the table. Nearly all figures of the table represent duplicate determinations made at intervals of one day to three weeks, and—in most cases—these determinations checked to within 10 mgms. Data were also obtained for suprarenal size at the time each bird was killed, but since only 8 of the 20 females used were entirely free from Ascaridia (none obviously tubercular) these data throw little further light on the relation of suprarenal size to ovulation; for, in the work referred to above[1] it was shown that the suprarenals of birds thus infested are usually continuously enlarged, are probably diseased, and do not show any definite hypertrophy at ovulation.

From 14 of the 20 females comparisons were obtained of the mid-ovulation stage with a stage more or less removed from ovulation. Of these 14 tests 12 show unquestionably higher values for the mid-ovulation stage than for stages more remote from the ovulation period. Moreover, both of the two exceptions proceed from samples taken at periods so far removed from both the previous and succeeding ovulations (see table) as to make it possible that instead of being really remote they may each represent a stage immediately preceding an ovulation which was suppressed by the heart punctures by which these samples were obtained. Again, in one of these two cases the comparison made is not with a mid-ovulation stage, but with stages 36 and 12 (?) hours preceding ovulation. For the remaining 6 of the 20 birds determinations are available from only one stage—these birds failing to survive the heart-punctures. In each of these six cases the amount obtained indicates that the mid-ovulation stage had the higher sugar value if the average values obtained for all birds of its kind be taken as a standard (see table).

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[1] Loc. cit.

If curves be formed from the individual determinations, by placing them at their exact intervals with reference to ovulation, and a separate curve made for each of the three groups, it is found that the highest points fall within the mid-ovulation period in all of the three curves. The curve for the "scragglies" departs most widely from the curve earlier obtained for suprarenal hypertrophy by Riddle.[1] If another curve be formed from

COMPARISON OF AMOUNTS OF BLOOD SUGAR OBTAINED WITHIN, AND APART FROM, OVULATION PERIODS.

| No. of Bird. | Periods with Reference to Ovulation and Amount of Blood Sugar. | | | |
|---|---|---|---|---|
| | Mid-ovulation. | Within 36 Hrs. | More than 36 Hrs. | |
| V65..... | 215 | — | 175 | Common pigeons |
| M386.... | 228 | — | 158 | |
| V27..... | 185 | — | *228* (6–5)[1] | |
| V270.... | 235 | — | 180 | |
| Ave.... | *216* | | *185* | |
| V90..... | — | 143 | *165* (4½–5)[1] | "Scraggly" common pigeons |
| V241.... | 230 | — | 178 | |
| V299.... | 200 | 130 | 149 | |
| V288.... | 173 | 143 | 145 | |
| Ave.... | *201* | *139* | *160* | |
| T149.... | 178 | — | 153 | Ring doves |
| P715.... | 180 | — | 135 (4½–5½)[1] | |
| T320.... | 175 | — | 155 | |
| T171.... | 198 | — | 145 | |
| T153.... | 153 | — | 123 | |
| T289.... | 150 | — | 130 (6–5)[1] | |
| Ave.... | *172* | — | *140* | |
| V205.... | 220 | — | — | Common pigeon |
| V28..... | — | 120 | — | Scraggly common pigeon |
| V260.... | — | — | 160 | |
| T282.... | 185 | — | — | Ring doves |
| T390.... | — | 155 | — | |
| T226.... | — | 160 | — | |

These determinations made at intervals so far removed (4.5 or more days) from both earlier and later ovulations as to leave it uncertain whether ovulation was in fact far removed or quite imminent—and a resorption of the ovum produced by the heart-puncture employed to obtain the sugar sample.

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[1] Loc. cit.

the data obtained from the five Ascaridia-free ring doves it is found to be the smoothest of the series and quite similar in its several parts to the curves for suprarenal hypertrophy and oviducal growth found in that earlier work. These several series of measurements therefore seem definitely to show that coincident with ovulation in the pigeon there occurs an increase of the blood sugar to 25 per cent. or more above the pre-ovulation value.

The results indicate: (1) That the stage with reference to ovulation is probably a factor influencing the values obtained for blood sugar in other animals and is probably too large a factor to be left out of consideration in dealing with samples taken from reproducing females. (2) That, the essential similarity of the curve expressing the rise of the blood sugar with the curve expressing the coincident hypertrophy of the suprarenals, as earlier reported by one of us, is a further evidence for the relationship of the suprarenals to carbohydrate metabolism on the one hand and to sexual functions on the other. (3) That the enforcement in pigeons of frequent and continuous ovulations throughout the year, as this has been practised and reported by Whitman and by Riddle—with important results on sex, viability, and longevity of offspring—is doubtless accompanied by an increased and nearly continuous mobilization of carbohydrate in the female parents.

### 185 (1932)

## Seasonal tide of blood phosphate in infants.

By **ALFRED F. HESS** and **MARION A. LUNDAGEN.**

[*From the Home for Hebrew Infants, New York City.*]

In a previous communication it was shown that in infants the inorganic phosphate of the blood could be raised to the normal level by frequent exposures to the sun's rays.[1] The same result can be brought about by means of the carbon arc-lamp. The most effective radiation in bringing about this alteration are the ultraviolet rays. As has been shown by Dorno, the rays of the sun during the summer are incomparably richer in ultraviolet than those during the winter and early spring. It seemed, therefore, of interest to follow, month by month, the content of inorganic phosphate in the blood of a group of infants, where dietetic and hygienic conditions could be controlled. The accompanying tables indicate the result of a systematic study of this kind. Table I shows that in June and July of last year the average inorganic phosphate of the blood of these infants, who varied in age from about 6 to 18 months, was 4.35 mg. per cent. During the summer and fall analyses were not carried out. In December the average percentage had fallen to 3.92 mg., and then decreased steadily during January, February and March. In April, especially during its latter half, and in the first half of May, the inorganic phosphate again began to rise. These averages represent some 270 individual tests. Table II shows that the high percentages in the summer and the fall, and the steady and marked ebb during the winter months, occur quite irrespective of the nature of the diet. This seasonal tide of the blood phosphate is to be attributed mainly to the seasonal variation of sunlight. It would seem that the ultraviolet rays are necessary for the normal metabolism of the growing infant, especially in relation to its exchange of inorganic salts.

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[1] Hess, A. F., and Gutman, P., PROC. SOC. EXPER. BIOL. AND MED., 1921, xix, 31.

TABLE I.

SEASONAL TIDE OF BLOOD PHOSPHATE (INORGANIC).

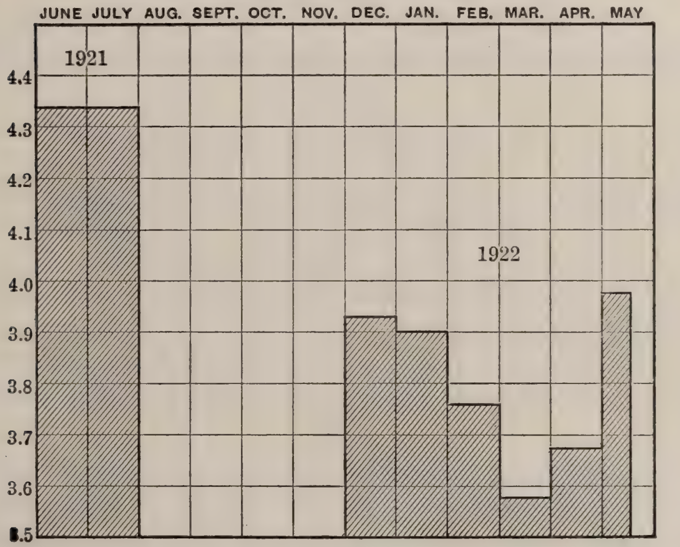

TABLE II.

SEASONAL TIDE OF BLOOD PHOSPHATE (INORGANIC).

| | 1921 June July | Dec. | Jan. | Feb. | Mch. | Apr. | May ½ Mo. |
|---|---|---|---|---|---|---|---|
| Protein M. | 4·45 | 3·91 | 3·86 | 3·64 | 3·48 | 3·45 | 3·95 |
| Dry M. | 4·21 | 3·87 | 3·77 | 3·68 | 3·30 | 3·46 | |
| Raw M. | 4·33 | 3·98 | 3·83 | 3·81 | 3·52 | 3·67 | |

### 186 (1933)

## Localized lesions in the corpora striata produced by buried radium emanation.

By D. J. EDWARDS and H. J. BAGG

[*From the Department of Physiology and the Memorial Hospital, Cornell University Medical College, New York City.*]

When a small glass capillary tube, containing about one millicurie of radium emanation, is imbedded in living tissues definite localized destructive reactions occur, mainly due to the relatively intense beta-ray radiation given off in the immediate vicinity of the tube.

Bagg has shown that when normal brain tissue is thus treated, and examined after a period of about two weeks, the tissue about the tube is completely necrotic, and is surrounded by a broad zone of polynuclear leukocytes beyond which there is some hyperemia. The entire lesion is limited to an area 1 cm. in diameter. The lesion is well localized, the brain cells beyond the one-centimeter zone retain their normal morphological character. That the lesion is practically entirely due to the irradiation of the radium emanation, and not to the presence of the glass tube, or the traumatism incident to its insertion, has been satisfactorily proven by control experiments.

The lesion just described is not produced at the time the small tube is placed in the tissues. From previous data obtained by examining various radiated areas at different periods after the time of insertion, it was found that the lesion slowly increased in size. At the end of 24 hours the zone of necrosis about the tube was only 1 mm. wide, and the maximum area of destruction (1 cm. in diameter) was reached at about the end of two weeks. The radium emanation loses its activity at the rate of 16 per cent. per day, and in clinical usage the tubes, when used as in this method, are said to have an effective period of irradiation equal to 132 hours.

In our experiments similar slow-growing, localized lesions were experimentally produced in the corpora striata of normal adult dogs. It was our aim to produce such lesions and to study the general physiological, and especially the neurological reactions that might occur as a result of such destruction. Although we were unable to produce at will the well-known clinical symptoms associated with lesions of the lenticular or caudate nuclei, our results are of interest in showing that an extensive amount of destruction of the basal ganglia is compatible with apparently normal neurological reaction, and that the organization of the brain is associated with marked compensatory ability.

Well-grown dogs were used as subjects. Morphine and novocain were used for anesthesia. The radium emanation tubes were from 2.5 to 3.5 mm. in length, about 0.4 mm. in diameter and with walls about 0.1 mm. thick. Each tube contained on the average one millicurie of radium emanation.

After anesthesia, a small incision was made in the scalp of the treated animal, about one centimeter lateral to the median line of the head, and just above the region of the coronal suture. A 2 mm. hole was drilled in the skull at about the intersection of the superior temporal line with the coronal suture. Through this hole the radium tube was inserted to the proper depth by means of a long, fine steel trocar, 0.8 mm. in outside diameter. The tube was left permanently in place.

Fifteen dogs were treated. In six, unilateral destruction of portions of the corpus striatum was performed, and the animals were killed at varying periods, the maximum being about 8 months after the operation. Four dogs were treated similarly, but at the end of 2, 3 and 4 weeks respectively a similar lesion was produced on the opposite side of the brain. In five animals a simultaneous bilateral destruction was made of portions of the corpus striatum on each side of the brain.

Our results have mainly shown that considerable portions of the corpora striata of dogs may be destroyed by our method without producing marked neurological disturbances. Following considerable destruction of the caudate and lenticular nuclei we have seen animals remain apparently normal for several months. This has occurred even when portions of the nuclei on each side of the head were simultaneously destroyed. However, three to five days following the treatment we have noted in several cases, hypertonicity, tremors, and a certain clumsiness in locomotion. Following these symptoms we have noted prompt compensatory adjustment, so that within a short time the animal is apparently again normal and remains so.

Clinically, the function of the corpus striatum is believed to be the control of automatic associative movements, and we believe that the symptoms we observed in our dogs are manifestations of disturbances in that mechanism.

We believe that our method of destruction due to radium emanation will prove of considerable value to the study of localization of functions in the basal ganglia of the brain.

### 187 (1934)

## An hypothesis of the mechanism by which normal rhythm is restored in atrial fibrillation.

By **HUBERT MANN.**

[*New York City.*]

The abrupt change from atrial fibrillation or flutter to normal sinus rhythm has become rather familiar to us since the use of quinidin. In spite of our greatly improved knowledge of the mechanism of fibrillation and flutter the exact way in which normal rhythm supersedes these arrhythmias is still a matter of speculation. The most prominent explanation, offered by Lewis,[1] has recently been considerably modified[2] and is still rather unsatisfactory because it fails to take into consideration events that may be taking place in the region of the sinu-atrial node.

[1] Lewis, T., *Brit. Med. Jour.*, 1921, 514.

[2] Lewis, T., *Heart*, 192, ix, 55.

It is the opinion of the writer that an adequate and satisfactory explanation of the change to regular rhythm will include a consideration not only of changes in the condition of circus contraction, but also of events in the immediate neighborhood of the normal pacemaker. This becomes more evident when we remember that the change in rhythm takes place quite suddenly at a time when the rate of flutter or fibrillation has been slowed to approximately twice the normal sinus rate.

Furthermore, the experimental evidence that we now have confirms our view, suggested by the experiments of Lillie on conduction and our own records of the effect of quinidin on the human heart, that quinidin not only increases the refractory interval but also slows the conduction rate. In fact the conduction rate seems, at least in some cases,[2] to be much more markedly affected than does the refractory interval. This is quite at variance with previous explanations of the effect of quinidin.

It is more in accord with known facts to believe that quinidin restores normal rhythm in the fibrillating or fluttering atrium, not by prolonging the refractory period to such an extent that circus movement is no longer possible, but by decreasing the conduction rate and so slowing up the circus movement to such an extent that the rhythmic function of the atrial muscle can reassert itself. A review of our knowledge of events prior to the sudden change in mechanism will make this more evident.

There is a fair amount of experimental evidence that in the condition known as atrial fibrillation there is a continuous circuit or circus movement taking place in the atrium at a rate somewhere in the neighborhood of 500 per minute. It is clear that with such a circus movement stimulating the atrium approximately 500 times a minute the sinu-atrial node, with its normal rate of 60–100 per minute, has very little chance of obtaining control and thus the condition of fibrillation is self-perpetuating. If we give quinidin to a patient with atrial fibrillation we know that the rate of this circus movement is slowed very appreciably. It is obvious that if the circus movement could be made slower than the sinus rate the sinus, if normal, would regain control and normal rhythm would be established. A more careful consideration of the problem will reveal that it will not be necessary to slow the rate of the circus movement below the normal sinus rate in order to obtain proper conditions for the restoration of normal sinus rhythm. Let us take for example a heart in which the normal sinus rate is 100 per minute, and in which the rate of circus movement has been slowed by quinidin to about 190 per minute. Let us follow our circus contraction and observe the events that take place. At first we notice that the circus movement stimulates the atrial muscle in the region of the sinu-atrial node and stimulates the node itself. But the node probably has a much greater nerve supply than the ordinary atrial musculature and therefore can be more sensitive to nervous influence and more subject to changes in the refractory period. As we watch we see that the region of the node, being for an instant slightly more refractory than usual, has failed to respond to one circus stimulus. But the rhythm of the node itself is sufficiently fast so that before the next stimulus arrives the node itself inaugurates a contraction. Whether or not this stimulus inaugurated by the node has time to spread very far before the next circus stimulus arrives depends on the exact time relations of the circus movement and the normal pacemaker, but if the stimulus inaugurated by the pacemaker has progressed as far as the central path of the circus movement it is evident that the circus movement will be blocked and will therefore cease, giving way suddenly to normal sinus rhythm.

In addition to the possibility of interruption of the circus movement by the normal pacemaker we must consider also the possibility of interruption by atrial premature contractions of extranodal origin. Such atrial premature contractions or extrasystoles are frequently encountered in patients with irritable nervous systems, and especially in patients who are being treated with quinidin. The manner in which the circus movement might be interrupted by an extranodal center is similar to that just described. The very frequent occurrence of atrial extrasystoles in patients who respond to quinidin therapy impels us to allot considerable importance to the rôle of such extrasystoles in the restoration of normal rhythm.

To summarize we propose for consideration the view that the way in which quinidin restores normal rhythm in the fibrillating atrium is by slowing up the conduction rate to such an extent that either the normal pacemaker or some ectopic source is enabled to reassert its rhythmic function and thus interrupt the circus movement.

In conclusion it should be remembered that the hypothesis here given is different from, but not contradictory to the hypothesis of Lewis. Both hypotheses are valid. In fact it is probable that both mechanisms take place and so explain the two different ways in which atrial fibrillation returns to normal rhythm. We refer to the sequence with quinidin—*i.e.*, fibrillation, flutter, normal rhythm, and the sequence under digitalis of flutter, brief fibrillation, and normal rhythm.

### 188 (1935)

## The emetic action of antimony and potassium tartrate (tartar emetic).

By **SOMA WEISS** and **ROBERT A. HATCHER.**

[*From the Department of Pharmacology, Cornell Medical College, New York City.*]

Tartar emetic (antimony and potassium tartrate) induces vomiting reflexly through local irritation after its introduction into the stomach or duodenum. The portion of the duodenum lying immediately below the pylorus is more sensitive than the stomach. Concentrated solutions are more active than dilute solutions in inducing this reflex.

Tartar emetic does not cause emesis in the cat or dog, when it is applied directly to the vomiting center decribed by Thumas, and which lies in the floor of the fourth ventricle.

Intravenous injections of tartar emetic induce vomiting after varying intervals of time, largely dependent on the size of the dose. This emesis is not prevented by the removal of the gastro-intestinal tract, or by the removal of the celiac plexus and simultaneous cutting of the vagi below the diaphragm, but it is profoundly influenced by cutting the vagi in the neck, or paralyzing the vagus endings with atropin; it is apparently abolished by severing all nervous connection between the heart and centers by removal of the stellate ganglia and cutting the vagi in the neck, in the cat.

The investigation is being continued.

### 189 (1936)

## The effect of thyroidectomy in two sittings upon depancreatized, non-glycosuric, but hyperglycemic dogs.

By **G. A. FRIEDMAN** and **J. GOTTESMAN**.

[*From the Department of Clinical Pathology, College of Physicians and Surgeons, Columbia University, New York City.*]

Dogs almost always show an increase in bloodsugar contents from removal of comparatively small amounts of pancreatic tissue. Of six dogs in whom hyperglycemia without glycosuria became manifest after partial pancreatectomy, in two the right lobe was removed first and at a later date lobectomy on the other side followed. In three hyperglycemic dogs the right lobe was completely removed, but while performing the lobectomy on the left side a tiny piece of thyroid tissue was left in connection with the superior parathyroid. In one dog both lobes were removed at the same time. In all of them three parathyroids were left in situ.

In the completely thyroidectomized diabetic dog the blood-sugar became normal on the day following the last operation. On the third day tetany developed which was kept in check by intravenous injections of calcium lactate. The bloodsugar had remained normal. On the fifth day a severe attack of tetany developed. Calcium lactate injections were not tried and the animal died.

The results of these experiments are in accord with those previously reported: one-sided lobectomy does not check hyperglycemia, neither does partial ligation in glycosuric dogs. Complete thyroidectomy in the hyperglycemic dog brought the bloodsugar to normal as the same procedure in glycosuric dogs when tetany did not ensue or when it was checked by calcium lactate.

Some new points were brought out in those dogs in whom at a second lobectomy a minute fragment of thyroid tissue was left.

In all of them the bloodsugar dropped to normal after incomplete removal of the gland on the left side at the beginning, but it increased later and has remained high.

While their weights were, 9, 7, and 10.8 kilos, respectively, the former dog showed a gain in body weight of 2.6 kilos thirty-eight days after the last operation; the second one 0.6 kilo fifteen days after the last operation; and the third dog 0.4 kilo fourteen days after the last operation. They all gained the losses in body weight which followed pancreatectomy.

As these dogs showed after incomplete thyroidectomy, high bloodsugar and a tendency to adiposity, we believe, therefore, to have produced in these animals a condition resembling the prediabetic state in man, *i.e.*, hyperglycemia and increase in body weight.

### 190 (1937)

## A chemical method of assaying strophanthus preparations.

By ARTHUR KNUDSON and MELVIN DRESBACH.

[*From the Laboratories of Biochemistry and Physiology, Albany Medical College, Albany, N. Y.*]

The method is based on the same principles as one for digitalis recently reported by us.[1] It is a colorimetric method making use of Baljet's[2] reaction, which takes place between the active principles of strophanthus and dilute alkaline picrate solution, giving an orange red color.

The method consists of first decolorizing the tincture, or other solutions of strophanthus, with lead acetate and then removing excess lead acetate with sodium phosphate. The decolorized solution is then treated with an alkaline picrate solution; the characteristic color develops in 20 minutes. As a standard for comparison a Ouabain solution is used in which the color is developed in a similar manner.

In the table below are given the results of this method compared with those by the Hatcher and Brody[3] cat bioassay method.

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[1] Knudson, A., and Dresbach, M., *Jour. Pharm. and Exp. Therap.*, 1922, xix, 268. This method will be reported in full in the *Jour. Pharm. and Exp. Therap.*

[2] Baljet, Henry, *Schweiz. Apoth. Ztg.*, 1918, lvi, 71–73 and 84–88.

[3] Hatcher, R. A. and Brody, J. G., *Amer. Jour. Pharm.*, 1910, lxxxii, 360–372.

It will be seen that the agreement between these two methods is very close. The chemical method permits of a direct determination of the amounts of strophanthin.

ASSAY OF STROPHANTHUS PREPARATIONS.

| Spec. No. | Kind of Preparation. | Chemical Assay. | Biological. | |
|---|---|---|---|---|
| | | | Assay. | No. of Det. |
| | | mg. = c.u.[1] | mg. = c.u. | |
| 7 | Strophanthus, amorphous, Merck . . | 0.135 | 0.116 | 2 |
| 34 | Tincture strophanthus. . . . . . . . . . . | 7.8 | 7.6 | 2 |
| 42 | “ “ . . . . . . . . . . . . | 8.7 | 8.7 | 3 |
| 44 | “ “ . . . . . . . . . . . . | 1.95 | 1.78 | 5 |
| 45 | “ “ , hispidus seeds | 1.87 | 2.04 | 3 |
| 46 | “ “ , kombe seeds. | 3.06 | 2.85 | 4 |
| 47 | Infusion strophanthus hispidus . . . . | 1.26 | 1.24 | 3 |
| 48 | “ “ kombe. . . . . . | 2.88 | 3.08 | 3 |
| 47 | “ “ hispidus . . . . | 1.75 | 1.51 | 3 |
| 47 | “ “ one month old | 1.19 | 1.40 | 3 |

[1] c.u. = cat unit of Hatcher and Brody.

## 191 (1938)

## Regeneration in Spathidium spathula and Blepharisma undulans.

By **E. LUCILE MOORE** (by invitation).

[*From the Osborn Zoölogical Laboratory, Yale University,*
*New Haven, Conn.*]

In a study of regeneration of form and function at various phases in the life history of the infusorians *Spathidium spathula* and *Blepharisma undulans*, it has been determined that the power of restoring lost parts and of continuing normal existence is in all cases dependent upon the same factor, the nuclear content of the fragment. Individuals of known pedigree were cut transversely, and the behavior or the fragments observed until regeneration and division or regeneration and death had occurred. During the vegetative state, regenerative power is highly developed in both ciliates, but because of the more distributed condition of the nucleus in *Spathidium* than in *Blepharisma* relatively smaller fragments from either extremity of the former are capable of complete regeneration. All fragments which continue to divide contain both macro- and micronuclear material. It has been impossible to produce an amicronucleate race by artificial means.[1]

In *Spathidium*, where the two nuclear elements extend throughout the cell both during and after fission, there is no difference in the regenerative power at any peroid of asexual life. In *Blepharisma*, however, where large amacronucleate fragments may be obtained during the process of division, it has been found that temporary restoration of external organelles is possible in the absence of nuclear material, if the formation of a new peristome has already been started. Dedifferentiation immediately follows and death occurs in a few days. The presence of micronuclei in such fragments, moreover, is not sufficient for continued existence.

During conjugation, complete regeneration in *Spathidium* is less frequent than during asexual life. Portions containing the synkaryon regenerate and divide; other pieces remain unchanged or undergo only temporary restoration of form. Fragments obtained from starved *Spathidia* prior to encystment either fail to regenerate, or regain normal form and subsequently encyst.

### 192 (1939)

## The almond as a source of the A vitamin.

By MARY SWARTZ ROSE and GRACE MACLEOD.

[*From the Department of Nutrition, Teachers College, Columbia University, New York City.*]

Investigations by Cajori have shown that the almond furnishes proteins adequate for growth, reproduction, and the suckling of young, and the B vitamin in liberal amounts.[2] Coward and Drummond have reported an experiment on three rats, of fourteen days' duration, in which one gram per day of almond was added to a basal ration devoid of the A vitamin, which indicates that this amount of nut does not furnish a sufficient supply of A vitamin for growth of albino rats,[3] but so limited an experiment is hardly to be regarded as conclusive, especially in view of the fact that the animals did not eat the almond very readily.

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[1] Woodruff and Spencer, *Journ. Exper. Zoöl.*, 1922, Vol. 35.

[2] Cajori, *Jour. Biol. Chem.*, 1920, xliii, 583–606.

[3] Coward and Drummond, *Biochemical Journal*, 1920, xiv, 665.

In the present investigation, attempts were first made to feed a diet of ground unblanched almonds, supplemented by a suitable salt mixture and a little starch, the almonds constituting 81 per cent. of the diet by weight. The records of daily food intake show that this diet was eaten in about the same amounts as the control diet, and the average calorie intake was higher than for the control diet. However, young rats placed on this diet at the age of about six weeks made no gains in weight in six or seven weeks. The addition of 2 grams of fresh compressed yeast to the ration of each rat caused a slight temporary increase in weight, but no permanent gain.

Thinking that there might be some inhibiting factor in the integument, as reported by Cajori in the case of the pecan,[1] the nuts were blanched before grinding and supplemented as in the case of the unblanched almonds, with no better results.

It then seemed probable that the high fat in the diet was the disturbing factor, and a press cake containing about 35 per cent. fat was used in making up a diet with the same protein and salt content as the original almond diet. The animals remained in somewhat better condition on this diet, showing less roughness of coat, but they declined steadily in weight.

A basal ration was then prepared consisting of meat residue, starch, lard, yeast and a suitable salt mixture, and containing the same percentage of protein as the almond diet. When young rats were fed this ration until they ceased to increase in weight (about four or five weeks), and then put on the same plus three per cent. of the blanched almond diet, they immediately began to gain in weight, and continued to grow well for five or six weeks. They then ceased to grow or declined in weight. Similar experiences were had with 5 per cent. blanched almond diet added to the basal ration. In case of three rats, increases to 10 per cent. of the almond mixture and later to 15 per cent. were followed by further growth. Considering the very good growth with only 3 per cent. of the almond mixture added to the basal diet, almonds would seem to be fairly rich in the fat-soluble A vitamin. The unfavorable effects of larger quantities of almond appear to be due to some harmful substance which is not in the integument but in the kernel itself. Since the results with press cake were almost identical with those from feeding the whole nut, it seems unlikely that the sole difficulty was the large amount of fat, though undoubtedly young animals will not thrive with a high amount of fat in the diet. We have repeatedly seen the coats of young rats look as if they had been dipped in water twelve to twenty-four hours after feeding almonds or peanuts. Unfavorable influences in the case of the peanut are also recorded by Daniels and Loughlin[1] and attributed to high fat.

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[1] Cajori, *Jour. Biol. Chem.*, 1921, xlix, 389–397.

### 193 (1940)

## A note on the use of ammonium chloride in gastric tetany.

By WILLIAM S. McCANN.

[*From the Chemical Division, Medical Clinic, Johns Hopkins Hospital, Baltimore, Md.*]

Studies of the experimental tetany produced by pyloric obstruction made by MacCallum[2] showed clearly a reduction in plasma chloride and a coincident increase in the $CO_2$ combining power of the blood plasma. A publication of a similar nature[3] called attention to this latter feature, but not to the reduction of plasma chloride content. The priority in experiments clearly belongs to MacCallum. Both reports agree in the interpretation that the primary disturbance is due to a loss of chloride through the gastric secretions. The therapeutic results obtained in experimental animals by replacing chloride ions either by injection of NaCl intravenously,[2] or HCl into the duodenum,[3] were good.

More recently attention has been called by Haldane[4] to the effects of administration of ammonium chloride by mouth. This substance when ingested produces an acidosis resulting in lowered $CO_2$ combining power of plasma, and an increased excretion of acid. The most probable explanation is that the ammonia is rapidly converted into urea leaving HCl to combine with other bases.

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[1] Daniels and Loughlin, *Jour. Biol. Chem.*, 1918, xxxiii, 295–301.

[2] MacCallum, Lintz, Vermilye, Leggett and Boas, *Bull. Johns Hopkins Hosp.*, 1920, xxxi, I.

[3] McCann, Wm. S., *Journ. Biol. Chem.*, 1918, xxxv, 553.

[4] Haldane, J. B. S., *Journ. Physiol.*, 1921, lv, 265.

In view of these facts it was determined to apply ammonium chloride to the treatment of gastric tetany. The opportunity presented itself in the case of Mrs. Helen G., aged 59 years, who had entered the hospital with a history of vomiting frequently for two years, and especially frequently during the preceding three months. An annular filling defect was noted in roentgenograms which was thought to be the result of carcinoma. A laparotomy performed revealed no evidence of carcinoma. Cholecystectomy was done for cholelithiasis. After operation vomiting continued. Five days later the typical carpopedal spasms of tetany were noted. A positive Trousseau's sign was obtained. Blood was taken for examination at this time. The patient was given an intravenous injection of 500 c.c. of an 0.822 per cent. solution of ammonium chloride, $P_H$ 7.0, which has been tested against the patient's blood and found to cause no hemolysis. Carpopedal spasms ceased during the injection and no return of tetany was noted up to the time of death twelve days later. The table shows the resulting changes in blood constituents.

| Date. | $CO_2$ C.P. Volume Per Cent. | Chlorides Whole Blood Mgs. per 100 c.c. | Non-Prot. Nitrogen Mgs. per 100 c.c. | Serum Calcium Mgs. per 100 c.c. |
|---|---|---|---|---|
| 4–13 before treatment.... | 75.7 | 386 | 30.6 | 8.8 |
| 4–13 ½ hr. after treatment | 53.0 | | | |
| 4–14.................. | 63.5 | 455 | 34.9 | |
| 4–21.................. | 58.2 | 484 | | |
| 4–23.................. | | 478 | 36.4 | |

Saline infusions were started after the second blood specimen was withdrawn, and continued daily until the 20th.

At autopsy there was found to be a diaphragmatic hernia containing the fundus of the stomach and producing a marked constriction. The esophagus entered so that practically only the fundus filled with food or bismuth.

Mention should be made of the recent successful use of ammonium chloride in the treatment of tetany in children.[1]

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[1] Freudenberg and György, *Klin. Wochenschr.*, 1922, i, 411.

194 (1941)

### The distribution of vitamin-B in the wheat kernel.

By **MARION BELL** and **LAFAYETTE B. MENDEL**.

[*From the Sheffield Laboratory of Physiological Chemistry, Yale University, New Haven, Conn.*]

Foods containing various percentages of wheat and wheat derivatives, and comparable to each other in their content of all known dietary essentials except such quantities of vitamin-B as might be present in the wheat product used were fed to mice; and the rate of growth on these foods was observed.

When the entire wheat kernel was thus used as the source of vitamin-B in the diet, admixtures of from fifteen per cent. (Marquis spring wheat), to forty per cent. (Minnesota winter wheat), were required to insure growth at a normal rate.

The approximate concentration of vitamin-B in each of the milling products from a single lot of winter wheat was estimated. The "patent flour" contained no appreciable vitamin; the "first clear" and "second clear" displayed about the same concentration as the unmilled grain; the "low grade flour" and "bran" were about twice as rich; the "standard middlings" (which included the portion containing most of the embryo) were four times as rich as the entire grain.

"Hand-dissected" portions of grains, representing more nearly the true *structural* divisions of both spring and winter wheat were also investigated. Vitamin-B was found in both embryo and endospern. The *concentration* in the embryo was several times as great as that in the endosperm; but owing to the small percentage of the entire kernel represented by the embryo, the *absolute quantity* of vitamin-B contained therein was not more than a sixth of the total amount in the grain. No difference could be detected between "hand-dissected" and "commercial" embryos. The presence or absence respectively of the embryo in the two ends of the grain, when fed separately, did not appreciably affect the concentration of vitamin-B in these two portions.

195 (1942)

## Rhythms in the rate of reproduction of Amœba bigemma.

By **E. FRANCES BOTSFORD** (by invitation).

[*From the Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.*]

A specimen of *Amœba bigemma* (Schæffer) was isolated December 7, 1921, and eight lines of its descendants have been bred in pedigree cultures to the 100th generation (May 15, 1922). The cultures have been carried on standard beef extract[1] and kept at a temperature varying from 20° to 24° C. Vegetative division has been uninterrupted by spore or gamete formation. The data obtained by the daily isolation method show that the rate of division of the organism under the conditions of the experiment fluctuates considerably. At intervals during the pedigree there were daily divisions, or frequently one division every other day for a short time. Occasionally two fissions occurred in one day, while in a single instance an *Amœba* divided three times during twenty-four hours. Periods of inactivity when no fissions took place were common, and varied in length from one to twelve days; but whereas the absence of division for one or two days was of frequent occurrence, that of over three days was rare. An average of the daily divisions in eight lines for a period of ninety days showed the rate to be slightly above one division in two days.

In contrast to this daily irregularity there was a general periodicity in the fission rate which was revealed only by an examination of the data covering longer intervals of time. By averaging the number of divisions in a single line for five-day periods, a rhythmic character of the fission rate was apparent in the form of alternating periods of high and low reproductive activity; one low point to the next comprising about twenty days. Whether these rhythms are of the same nature as those in the Infusoria[1] can be determined only after more study of this and other *Amœbæ* in pedigree cultures.

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[1] Woodruff and Baitsell, *Journ. Exper. Zoölogy*, 1911, Vol. 11.

### 196 (1943)

# The influence of light on the toxicity of quinidin and quinin sulphates.

By **D. I. MACHT.**

[*From the Pharmacological Laboratory of the Johns Hopkins University, Baltimore, Md.*]

It is well known that solutions of quinin and quinidin sulphates are fluorescent. This fact suggested an inquiry into whether such solutions are as toxic in the dark as when exposed to light. Accordingly the present investigation was undertaken to settle this question. Such an inquiry was deemed to be worth while especially in view of the older observations by H. Tappeiner and O. Raab in 1900,[1] who noted that solutions of the dye *acridin* were much more toxic for paramœcia in sunlight than in the dark. The present author began his studies on frogs. An *aqueous* solution of quinidin sulphate was made. Various doses of the same were injected into the anterior lymph sacs of frogs and the effects of the drug were studied by exposing some frogs to light while keeping others in darkness. It was found that the quinidin solution was much more toxic when animals were exposed to sunlight than when they were kept in the dark. Thus for instance in one experiment a dose of quinidin sulphate, 0.5 mgm. per gm. weight, was injected into the anterior lymph sac of a *rana clamata*. Exactly the equivalent dose of quinidin from the same solution was injected into a second frog in the same way. The first frog was exposed to sunlight. It was found to be paralyzed 25 minutes after injection, and the heart was found to have been arrested completely 30 minutes after injection. The second frog was placed in a dark cupboard and was still alive 24 hours after the beginning of the experiment. Similar experiments with varying doses of quinidin were performed many times and a comparison of the data thus obtained clearly indicated that the toxicity of the drug was much greater when the frogs were exposed to sunlight. Insamuch as a difference in temperature may affect to some extent the condition of frogs, especially as regards their central nervous system, control experiments were made to determine whether the above differences were due to the differences in temperature. This was shown not to be the case: it was the illumination and not the temperature which rendered the drug more toxic. Further experiments revealed that it was the light waves from the violet end of the spectrum that were the most effective in increasing the toxicity of quinidin. In still other experiments the rays of an electric arc lamp were utilized instead of sunlight and the same potentiation in toxicity was qualitatively noted. Finally a few experiments on excised frogs' hearts were performed and the results obtained so far have maintained the above findings.

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[1] *Zeit. f. Biologie,* 1900, xxxix, 37.

In the above experiments an aqueous solution of quinidin was used because quinidin solutions are known to lose much of their fluorescence when alkalis or even sodium chloride are added to them. Tests made however by adding various amounts of blood serum and even sodium chloride to solutions of quinidin sulphate indicated that while these decreased the fluorescence markedly they did not abolish it completely; and it was further found that even such poorly fluorescent substances showed the difference in toxicity as between light and darkness described above.

Quinin solutions were found to behave in much the same way as those of quinidin. It may also be added that the toxic effect on frogs appears much more rapidly if the animals are placed in such a position as to allow the sunlight to fall directly on the white unpigmented skin of the abdomen, instead of the pigmented skin of the rest of the body. Further and more extensive work on the subject is in progress and will be continued. The present note is published as a preliminary communication and in order to fix priority of date of discovery.

### 197 (1944)

## The therapeutic effect of germanium dioxide in anemia.

By LUDWIG KAST, HILDA M. CROLL and HERBERT W. SCHMITZ.

[*From the Departments of Medicine and Biochemistry, New York Post-Graduate Medical School and Hospital, New York City.*]

The erythropoietic action of germanium dioxide in animals and in one normal man was demonstrated by Hammett and

Müller.[1] We have treated ten patients suffering from anemia, administering the germanium (N. J. Zinc Co. product) by mouth in 0.2 per cent. water solution. The dosage was between 100 and 200 mgs. of germanium dioxide given daily, or in some cases every two or three days, until between 950 and 1,400 mgs. had been given. In three cases of anemia following hemorrhage there were maximum increases in the number of erythrocytes per cubic millimeter of blood amounting to 77.2, 71.4 and 41.5 per cent. above the control counts. The hemoglobin in these cases increased to a maximum of 53.2, 35.1 and 56.7 per cent. above the controls. In addition to the germanium, one and one half ounces of "ovoferrin" were given daily to the last patient, who had carcinoma of the uterus. The condition of the patient became worse, however, and she died on the day of the maximum red-cell count.

In five cases of secondary anemia, with diagnoses including visceroptosis, colitis, tachycardia, carcinoma of the breast and malignant endocarditis, after treatment with germanium the red cells increased to a maximum of 23.4, 26, 53.4, 25.8 and 5.4 per cent. above the control counts, with increases in hemoglobin up to 9.5, 10.1, 14.5, 20 and 3.1 per cent. above the controls. In one of these cases after the increase in red cells of 53.4 per cent. there was a drop to 40 per cent. above the control one week after the last dose of germanium, the count remaining at this level for about ten days and then dropping to 7.7 per cent. above the control. A second treatment with several doses of germanium resulted in increases up to 24.5 per cent. above the control count.

In a man suffering from chronic cardiovalvular disease there were small increases and decreases over the control red-cell counts, and decreases as great as 15.9 per cent. below the control hemoglobin content after the germanium treatment. It appears that in this case the medication was of no value.

A woman with carcinoma of the cervix showed decreases in both red cells and hemoglobin after germanium. This patient had been treated with radium one week before the first dose of germanium.

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[1] Hammett, F. S., Nowrey, J. E. and Müller, J. H., *J. Exp. Med.*, 1922, xxxv, 173. Müller, J. H., and Iszard, M. S., *Am. J. Med. Sci.*, 1922, clxiii, 364. Hammett, F. S., Müller, J. H. and Nowrey, J. E., *J. Pharm. Exp. Therap.*, 1922, xix, 337.

In all of the cases studied there was no significant change in the white blood-cell counts. Judging from the percentages of blood total solids, the increases in red blood cells were not due to a concentration of the blood from loss of fluid. The color index generally dropped early in the treatment with a gradual rise later. A study of chemical blood and urine analyses in certain cases revealed no apparent effect of the germanium on the functional activity of the kidneys. The periodic effect on the red-cell count noted by Hammett and Müller was also noted in this study.

### 198 (1945)

## Influence of ischemia on infection.

By **TORALD SOLLMANN** and **J. G. BRODY.**

[*From the Department of Pharmacology of the Medical School of Western Reserve University, Cleveland, Ohio.*]

The following experiments furnish a striking illustration, suitable for class-room demonstration, of the influence of temporary ischemia on the local resistance to infection:

Two or three slight cuts are made on the upper or middle third of each ear of a rabbit, by closely clipping the hair from the dorsal surface of the ear; lifting with forceps a small fold of the skin, and snipping this fold away with scissors. This makes small wounds of about 4 by 7 mm., usually without hemorrhage. The wounds may then be smeared with active agar-cultures of staphylococcus or pyocyaneus; or they may be left without artificial infection. Within a few minutes after making the wounds, 1 c.c. of epinephrin, 1 : 1000, is injected into the root of one ear, close to the entrance of the vessels. This produces an intense ischemia of the entire ear, persisting for several hours.[1]

From the following day, the two ears present a striking difference in appearance: The wounds on the normal ear show signs of healing, even if they were severely infected. The wounds on the ear that had been rendered anemic appear much more inflamed, and may be covered with pus, and a perforating ulcer may have started. This is shown in the adjoining photograph, taken 24 hours after inoculation with a virulent staphylococcus culture, recently prepared from a human throat: The epinephrin ear (the right) shows the perforation of the ear by the infection; whilst in the left ear, no inflammation is visible on the inner side, although the ear had been equally wounded and inoculated, but not treated with epinephrin. These differences persist in the further course; the ear that had received epinephrin healing more slowly than the other ear.

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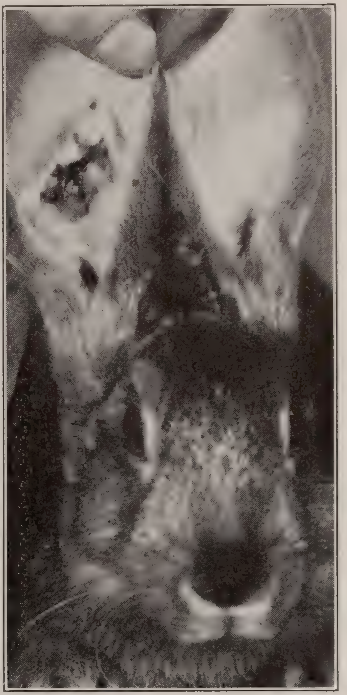
[1] Auer and Meltzer, *Proc. Soc. Exper. Biol. and Med.*, 1916, xiv, 54.



Marked differences, although not always quite as striking as these, were obtained in five inoculated rabbits, and in four rabbits without artificial inoculation. In only one rabbit did there fail to be a striking difference between the two ears.

Injection of slightly acidulated water, on the other hand, did not influence the course, showing that the difference is due to the epinephrin, and not to the volume or reaction of the fluid.

The experiment illustrates the current conceptions as to the origin of "colds," *i.e.* by increased susceptibility to infection, through the reflex vasoconstriction of "chills." It also warns against the indiscriminate local use of epinephrin in wounds, and especially in catarrhs.

### 199 (1946)

## Morphology of cystic growths in the ovary and uterus of the guinea pig.

By G. N. PAPANICOLAOU and C. R. STOCKARD.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

The ovary in the guinea pig, as in most other mammals, frequently presents a cystic condition. The cysts are the result of a proliferation of the cuboidal epithelium which lines the epididymal portions of the embryonic Wolffian duct. The epididymal tubules are located towards one pole of the ovary and are connected with similar tubules lying outside the body of the ovary between it and the oviduct.

Under certain conditions the walls of these blind tubules begin to proliferate, apparently forming a number of new tubules. A fluid accumulates in the interior of the tubules and distends them into spheroidal shapes. They become greatly distended and break into one another or fuse, thus forming large "ovarian cysts" in the case of those tubules lying within the ovary or "parovarian cysts" in the tubules lying outside.

Thus the ovarian and parovarian cysts are similar in structure and their formation is of the nature of a tumor-like growth of the cuboidal epithelium which lines them. The accumulation of fluid which is essential to the formation of typical cysts is not to be considered their primary cause.

In studying a great many ovaries for cystic conditions during several years we have never observed a follicular cyst. Large atretic follicles may be confused at times with small cysts, but such follicles always begin to disappear or atrophy before attaining significant dimensions.

The uterine glands occasionally become cystic. Such cysts usually break into the lumen of the uterus when their epithelial lining becomes greatly distended. These are similar to the ovarian cysts in that both occur under identical conditions in tubules lined by epithelium. The fact that the uterine glands open directly into the lumen of the uterus makes the occurrence of such cysts exceptional.

### 200 (1947)

## Experimental results bearing on the etiology of cystic growths in the ovary and uterus of the guinea pig.

By G. N. PAPANICOLAOU and C. R. STOCKARD.

[*From the Department of Anatomy, Cornell University Medical College, New York City.*]

In experiments on underfeeding it was found that malnutrition readily gave rise to marked cystic conditions in the ovaries of healthy young guinea pigs. Such cystic conditions are, of course, frequently found in normal stock but here especially in old or unhealthy specimens.

The changed nutritive conditions in the reproductive organs of underfed animals cause circulatory congestion, and as was pointed out in a previous communication[1] such conditions suppress the œstrous changes and prevent ovulation in these animals. The congestion and the high pressure resulting therefrom seem to favor the proliferation of the epithelial lining of the epididymal tubules located near one pole of the ovary, and the accumulation of fluid within the lumen of the blind tubules.

The malnutrition expresses itself first within the ovary by a wholesale degeneration of developing follicles which seem to respond most delicately to changes in nutritive conditions. The congestion and follicular degeneration seem then to favor an overgrowth of the more resistant epididymal tubules which become distended and crowd out the parenchymatous portion of the ovary.

Uterine cysts seem to develop in the same way as those above as a response to the congestion resulting from malnutrition. The open mouths of the uterine glands make their cystic condition rare so that among hundreds of ovarian cysts of all sizes we have observed only one perfectly typical case of uterine cyst.

These experiments seem to indicate that ovarian and parovarian cysts represent growths of persistent embryonic tissue, and that an accompanying congestion and high pressure are necessary to the formation of typical cysts, and that these conditions may result from disturbed nutrition as is demonstrated by underfeeding the guinea pigs.

## FROM THE PACIFIC COAST BRANCH.

201 (1948)

### The synthesis of benzoyltaurin.

By **CARL L. A. SCHMIDT** and **W. E. SCOTT**.

[*From the Department of Biochemistry and Pharmacology of the University of California, Berkeley, Cal.*]

It appears to be a specific function of several of the amino acids to combine with certain substances which the body is unable to oxidize. When benzoic acid and the numerous aromatic substances which are converted into benzoic acid in the body are ingested by man and certain other animals they are eliminated in the urine almost quantitatively as hippuric acid. Phenylacetic acid is voided in the urine of the dog and of the rabbit conjugated with glycocoll as phenaceturic acid[1] while in man it combines with glutamine and appears in the urine as phenylacetylglutamine.[2] The following also belong to the list of substances which undergo conjugation with glycocoll in passing through the body: furfurol[3] which in part is first oxidized to pyromucic acid and excreted as pyromucuric acid and in part combines with acetic acid and is eliminated as furfuracryluric acid, $\alpha$-methylthiophene[4] which is oxidized to $\alpha$-thiophenic acid and appears in the urine as thiophenuric acid, and $\alpha$-methyl pyridin ($\alpha$-picolin)[5] which after oxidation to $\alpha$-pyridin carboxylic acid passes into the urine as $\alpha$-pyridinuric acid. The rôle which in animals is given to glycocoll is taken in birds by ornithin.[6] Ingestion of benzoic acid by chickens leads to the appearance of ornithuric acid and similarly, pyromucic acid is conjugated with ornithin to give pyromucinornithuric acid. In certain instances the part of the conjugating amino acid is taken by cystein[7] and by taurin. Halogen combinations of benzol and naphthalin are linked in the body with cystein and this substance, after acetylation of the amino group, appears in the urine combined with glucuronic acid. In the bile of most animals both glycocoll and taurin are found in combination with cholic acid although in the bile of the dog and of the sheep taurocholic acid appears to be the only bile salt present.[1]

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[1] G. N. Papanicolaou and C. R. Stockard, PROC. SOC. EXP. BIOL. AND MED., 1920, xvii, 143.

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[1] Salkowski, E., *Z. physiol. Chem.*, 1885, ix, 229.

[2] Thierfelder, H., and Sherwin, C. P., *Berichte*, 1914, xlvii, 2630. Thierfelder, H., and Sherwin, C. P., *Z. physiol. Chem.*, 1915, xciv, 1. Sherwin, C. P., Wolf, M., and Wolf, W., *J. Biol. Chem.*, 1919, xxxvii, 113. Shiple, G. J., and Sherwin, C. P., *J. Amer. Chem. Soc.*, 1922, xliv, 618.

[3] Jaffé, M., and Cohn, R., *Berichte*, 1887, xx, 2311.

[4] Jaffé, M., and Levy, H., *Berichte*, 1888, xxi, 3458.

[5] Cohn, R., *Z. physiol. Chem.*, 1893, xviii, 112.

[6] Jaffé, M., *Berichte*, 1877, x, 1925; 1878, xi, 406. Jaffé, M., and Cohn, R., *Berichte*, 1888, xxi, 3461. Totani, G., *Z. physiol. Chem.*, 1910, lxviii, 75. Suga, T., *Chem. Abstr.*, 1921, xv, 881.

[7] Baumann, E., and Preusse, C., *Berichte*, 1879, xii, 806. *Z. physiol. Chem.*, 1881, v, 309. Baumann, E., and Schmitz, P., *Z. physiol. Chem.*, 1895, xx, 586. Jaffé, M., *Berichte*, 1897, xii, 1092.

No adequate theory has been advanced to explain the specific rôle which is thus played by these amino acids. It cannot be entirely a question of availability since probably all of the amino acids appear in the blood stream. The facts at hand are still too few to warrant an hypothesis. An attempt was made a number of years ago by Koelker and Amberg[2] to influence the normal course of the synthesis of hippuric acid. On simultaneous administration of benzoic acid and dl-leucin, benzoyl leucin did not appear in the urines of the experimental rabbits while the presence of hippuric acid and free benzoic acid was easily shown. The authors conclude that dl-leucin possesses a detoxifying effect on the action of benzoic acid although no explanation is offered as to the mechanism of this action.

## ANIMAL EXPERIMENTS.

It appeared to us that since taurin behaves in nearly all respects like an amino carboxylic acid[3] and that since both taurin and glycocoll are combined with cholic acid as bile salts, an opportunity was afforded for the possible substitution of taurin for glycocoll when benzoic acid is ingested. Taurin, when administered to the dog or to man, is not broken down but is excreted in the urine unchanged.[4] An opportunity is thus afforded not only for the possible conjugation with benzoic acid in the body but also for the estimation of uncombined taurin in the urine. Benzoic acid and taurin were simultaneously fed to both man and to dogs. The dose of the latter substance was several times that required to combine with the amount of benzoic acid which was given. In the experiments of the first series estimations of total nitrogen, amino nitrogen, free and conjugated benzoic acid (Folin and Flanders),[5] neutral sulfur, total sulfates and total sulfur were carried out. A portion of the urine was also used for the isolation of hippuric acid according to Dakin's method.[1] On account of the difficulty of removing pigments the method in our hands was not adapted for the quantitative estimation of hippuric acid but it did serve to establish the presence of this substance qualitatively. In the second series of experiments attempts were made to estimate benzoyltaurin directly. A volume of urine was evaporated to dryness and extracted with ethyl acetate in accordance with the procedure used by Dakin for the estimation of hippuric acid. After evaporating the ethyl acetate an estimation of neutral sulfur was carried out on the aqueous solution of the residue. The result of an experiment in which benzoyl taurin was added to normal urine indicated a recovery of 70 per cent.

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[1] Schmidt, C. L. A., and Dart, A. E., *J. Biol. Chem.*, 1921, xlv, 415.

[2] Koelker, A. H., and Amberg, S., *J. Pharmacol.*, 1910–1911, ii, 59.

[3] Schmidt, C. L. A., PROC. SOC. EXP. BIOL. AND MED., 1921, xviii, 204.

[4] Schmidt, C. L. A., Von Adelung, E. and Watson, T., *J. Biol. Chem.*, 1918, xxxiii, 501. Schmidt, C. L. A. and Allen, E. G., *J. Biol. Chem.*, 1920, xlii, 55.

[5] Folin, O., and Flanders, F. F., *J. Biol. Chem.*, 1912, xi, 257.

The data indicate that no appreciable synthesis of benzoyltaurin takes place in the body when taurin and benzoic acid are simultaneously administered. The increase in neutral sulfur (62 per cent. of the taurin sulfur) and in amino nitrogen (60 per cent. of the taurin nitrogen) of Subject No. 1 agrees with the results of former work on the output of taurin when ingested by man. The experiments carried out on dog No. 1 also indicates a recovery ($S = 59$ per cent., $NH_2 = 66$ per cent.) or about 60 per cent. of the dose of taurin which was given. The figures for neutral sulfur in the experiments of series II are within the limits of the normal variability and indicate that benzoyltaurin was not present in amounts which can be estimated. The figures for benzoic acid indicate that this substance was almost quantitatively recovered. The presence of hippuric acid in the urine was established in all of the experiments.

## SYNTHESIS OF BEZOYLTAURIN.

Baum[2] who prepared the benzoyl derivatives of a number of the amino acids by treating them with benzoyl chloride reports that he was unable to similarly synthesize benzoyltaurin. Later the substance was prepared by Gabriel and Heymann[3] from

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[1] Dakin, H. D., and Hawk, P. B., "Practical Physiological Chemistry," Philadelphia, 1918, p. 543.

[2] Baum, J., *Z. physiol. Chem.*, 1885, ix, 465.

[3] Gabriel, S., and Heymann, P., *Berichte*, 1890, xxiii, 157. Gabriel, S. and Colman, J., *Berichte*, 1911, xliv, 3628.

$\mu$-phenylthiazolin. In the synthesis of benzoyltaurin use was made of a buffer substance such as $NaHCO_3$ or $NaOOCCH_3$ instead of NaOH which was employed by Baum to neutralize the HCl which is set free during the course of the reaction. The procedure follows essentially the method used by Fischer[1] for the benzoylation of other amino acids.

Five grams of taurin in solution were shaken with 10 grams of benzoyl chloride in the presence of sufficient $NaHCO_3$ to keep the reaction slightly alkaline. The benzoylation was continued until no nitrogen was obtained when a test portion was treated with $HNO_2$. The solution was made just acid by addition of HCl, cooled in the ice chest and the benzoic acid was removed by filtration. The filtrate was evaporated to dryness at a low temperature and the residue was extracted with petroleum ether to remove traces of benzoic acid. The residue was then extracted with dry ethyl acetate in which solvent benzoyltaurin is slightly soluble. Unlike hippuric acid and other benzoylated amino acids benzoyltaurin is not easily crystallizable either in the free state or as the sodium salt, hence purification is difficult. It is very soluble in water and only slightly soluble in alcohol. Analysis of our product gave the following values:

| | Found. | Calculated for $C_6H_5COHNCH_2CH_2SO_3Na$. |
|---|---|---|
| Nitrogen | 5.5% | 5.6% |
| Sulfur | 13.4% | 12.8% |
| Sodium (as $Na_2SO_4$) | 9.8% | 9.2% |
| $\alpha$-$NH_2$ | 0.3% | —— |

TABLE I.

THE SIMULTANEOUS ADMINISTRATION OF SODIUM BENZOATE AND TAURIN.

| Dose. | $\alpha$-$NH_2$, Mg. | Total Sulfur, Gm. | Total Sulfates, Gm. | Neutral Sulfur, Gm. | Total Nitrogen, Gm. | Free Benzoic Acid, Gm. | Conjugated Benzoic Acid, Gm. |
|---|---|---|---|---|---|---|---|
| *Dog. No. 1.* | | | | | | | |
| | 68 | 0.152 | 0.082 | 0.070 | 2.8 | | |
| 6 gm. taurin, 6.8 gm. benzoic acid | 330 | 1.155 | 0.092 | 1.063 | 3.5 | 0.58 | 5.82 |
| | 187 | 0.260 | 0.016 | 0.144 | 3.2 | | |
| | 71 | 0.144 | 0.041 | 0.103 | 3.2 | | |

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[1] Fischer, E., *Berichte*, 1899, xxxii, 2451.

*Human Subject*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| .......................... | 141 | 0.938 | 0.770 | 0.168 | 13.6 | 0.11 | 0.44 |
| 8 gm. taurin, 3.76 gm. benzoic acid.................... | 692 | 2.360 | 0.888 | 1.472 | 14.4 | 0.10 | 3.56 |
| .......................... | 163 | 1.220 | 1.002 | 0.218 | 15.3 | 0.09 | 0.38 |

TABLE II.

THE SIMULTANEOUS ADMINISTRATION OF SODIUM BENZOATE AND TAURIN.

| Dose. | Free Benzoic Acid, Gm. | Conjugated Benzoic Acid, Gm. | "Neutral Sulfur," Gm. |
|---|---|---|---|
| *Dog. No. 2.* | | | |
| ................................ | 0 | 0.15 | 0.009 |
| 3.76 gm., benzoic acid................ | 0.19 | 3.36 | 0.032 |
| 3.76 gm. benzoic acid, 11.25 gm. taurin . | 0.77 | 3.43 | 0.013 |
| 9.04 gm. benzoic acid, 27.0 gm. taurin .. | 2.50 | 2.44 | 0.010 |
| ................................ | 1.61 | 2.32 | 0.007 |
| *Human Subject.* | | | |
| ................................ | 0.04 | 0.37 | 0.014 |
| 1.51 gm. benzoic acid................. | 0.05 | 1.50 | 0.014 |
| 1.51 gm. benzoic acid, 4.5 gm. taurin ... | 0.07 | 1.55 | 0.014 |

### 202 (1949)

## Blood-sugar studies.

By **G. L. FOSTER.**

[*From the Department of Biochemistry and Pharmacology of the University of California, Berkeley, Cal.*]

In a recently reported study of certain blood-sugar phenomena McLean and de Wesselow[1] have attempted to explain the nature of the blood-sugar curve obtained after the ingestion of glucose by man. As a result of the comparison of the curves of a normal and of a diabetic individual they suggest that the existence of an alimentary hyperglycemia in the normal awakens and stimulates the glycogen-forming mechanism to such activity that not only is the rising hyperglycemia checked but that the blood-sugar concentration is rapidly brought down to normal or below, thus accounting for the rapid rise and fall of blood sugar which is the characteristic response of the normal to the ingestion of glucose.

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[1] McLean, H. and de Wesselow, O. L. V., *Quart. Jour. Med.*, 1921, xiv, 103.

In the diabetic, on the other hand, the glycogenic function is impaired, it does not respond to the stimulus, and the blood sugar continues to rise as long as glucose is absorbed from the intestine.

Staub [1] in Spiro's laboratory, has offered a similar suggestion, the gist of which is that the presence of abundant carbohydrate food in the body stimulates the mechanism which is concerned with carbohydrate metabolism.

In a more recent paper Folin and Berglund[2] have found the hypothesis of McLean and de Wesselow to be superfluous. To them not glycogen formation but simply absorption into the tissues is entirely adequate to account for the fact that sugar does not accumulate in the blood after the ingestion of glucose or other sugars. In arriving at this conclusion they were apparently greatly influenced by their blood-sugar findings after feeding fructose and galactose to normal individuals. The latter is known to be a poor glycogen former, hence, if glycogen-formation is an important factor in reducing or preventing alimentary hyperglycemia, then galactose should be much more effective than glucose in raising the blood-sugar level. As a matter of fact, however, they found absolutely no rise after feeding galactose, nor after fructose and maltose. They make no mention of data in the literature contrary to their findings in this respect.[1, 2, 4, 5]

The purpose of this paper is to report in a very preliminary manner some data which favor the hypothesis of McLean. We think that our results with fructose, galactose and maltose (Tables I and II) remove the ground for Folin and Berglund's objection to McLean's interpretation. In four tests with Kahlbaum's purest galactose marked increases in blood-sugar concentration were found, in two cases extraordinary hyperglycemia developed, reaching a maximum in about two hours, which corresponds in time with the maximum glycosuria found by Folin and Berglund. Our maltose curves agree with the findings of Field,[3] McLean and de Wesselow[4] and Leire.[5] With fructose both Folin and Berglund and McLean and de Wesselow found no rise in blood-sugar level.

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[1] Staub, H., *Biochem. Zeits.*, 1921, cxviii, 93.

[3] Folin, O. and Berglund, H., *Jour. Biol. Chem.*, 1922, li, 213.

[3] Field, C. W., PROC. SOC. EXP. BIOL. AND MED., 1919, xvii, 29.

[4] Loc. cit., p. 408.

[5] Bang, I., "Der Blutzucker," p. 63.

TABLE I.

EFFECT OF INGESTION OF GALACTOSE.

| Time. | Blood Sugar. Mg. per 100 c.c. Whole Blood. | | | |
|---|---|---|---|---|
| | Exp. 8, 100 gm. Galactose. | Exp. 9, 100 gm. Galactose. | Exp. 17, 40 gm. Galactose. | Exp. 20, 60 gm. Galactose. |
| Before | 82 | 86 | 84 | 96 |
| 15 min. after | 139 | 101 | 154 | 147 |
| 30 " " | 160 | 102 | 154 | 163 |
| 45 " " | 170 | 123 | 117 | 160 |
| 75 " " | 235 | 179 | 93 | 132 |
| 105 " " | 263 | 206 | 82 | 101 |
| 135 " " | 290 | 247 | 90 | 97 |
| 165 " " | | | 88 | 104 |
| 180 " " | 186 | 161 | 90 | |

TABLE II.

EFFECT OF INGESTION OF MALTOSE AND OF FRUCTOSE.

| Time. | Blood Sugar. Mg. per 100 c.c. Whole Blood. | | | |
|---|---|---|---|---|
| | Exp. 22, 75 gm. Maltose. | Exp. 23, 100 gm. Maltose. | Exp. 25, 75 gm. Fructose. | Exp. 26, 90 gm. Fructose. |
| Before | 100 | 95 | 84 | 95 |
| 8 min. after | — | — | 101 | 125 |
| 15 " " | 144 | 157 | 108 | 126 |
| 25 " " | — | — | — | 128 |
| 30 " " | 89 | 165 | 105 | 111 |
| 45 " " | 91 | 118 | — | 101 |
| 55 " " | — | — | 92 | — |
| 70 " " | — | — | — | 104 |
| 85 " " | 77 | 91 | — | — |
| 120 " " | 104 | 101 | — | — |

It is well known that fructose is much more rapidly oxidized in the body than glucose[1, 2, 3] and there is some evidence that it is a better glycogen former.[4] Taking our blood samples at short and frequent intervals we could detect a rather small but unmistakable rise of blood sugar in two fructose tests.

In studying the effects of repeated doses of glucose we have obtained results quite different from those of McLean and de

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[1] Johansson, J. E., *Skand. Arch. Physiol.*, 1908, xxi, 1.

[2] Lusk, G., *Jour. Biol. Chem.*, 1915, xx, 555.

[3] Bürger, M., *Biochem. Zeits.*, 1921, cxxiv, 1.

[4] Weinland, E., *Zeits. f. Biol.*, 1899, xxxviii, 16 and 607.

Wesselow. They gave fifty grams of glucose which caused the blood sugar to rise and fall sharply. Then, when the curve had returned to normal, they gave a second dose of fifty grams which produced a response almost identical with the first. We find that the second dose may cause little or no response, depending upon the time at which it is taken (Table III). If the second dose is taken when the rise is near its peak it produces only a slight "bump" in the curve, which we interpret as meaning that the mechanism (glycogen formation?) has nearly attained the same speed as the inflow of sugar from the gut. However, if the second dose be taken soon after the curve has returned to normal or below, it has no detectable effect on the sugar content of the venous blood (*i.e.*, the mechanism is now at top speed and can handle any quantity of sugar pouring in from the intestine). McLean worked with "finger blood" (arterial and capillary) while we used venous. We cannot yet say whether or not this is the cause of the apparent discrepancy.

TABLE III.

EFFECT OF REPEATED DOSES OF GLUCOSE.

Two doses of 100 gm. each at interval as noted by asterisk.

| Time. | Blood Sugar. Mg. per 100 c.c. Whole Blood. | | | |
|---|---|---|---|---|
| | Exp. 21. | Exp. 24. | Exp. 27. | Exp. 4. |
| Before first dose | 95 | 99 | 100 | 109 |
| 15 min. after first dose | 171 | 139 | 166 | — |
| 30 " " " " | 140 | 81 | 115 | 150[1] |
| 45 " " " " | 102 | — | — | — |
| 60 " " " " | 105[1] | 62[1] | 84[1] | 156 |
| 75 " " " " | 101 | 57 | 85 | — |
| 90 " " " " | 95 | 63 | 88 | 99 |
| 105 " " " " | 86 | 57 | 89 | — |
| 120 " " " " | — | — | — | 78 |

[1] Second dose swallowed immediately after this blood sample was taken.

Finally, the epinephrine hyperglycemias are not to be reconciled with Folin and Berglund's hypothesis. Here absorption by the tissues is normal (Palmer)[1] but nevertheless the hyperglycemia is excessive and prolonged. We are led to the conclusion that the chief factor in preventing hyperglycemia is glycogen formation, since this is presumably the only carbohydrate function which is upset by epinephrine. May we not have a mechanism analogous to secretin which controls the glycogenic function?

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[1] Palmer, W. W., *Jour. Biol. Chem.*, 1917, xxx, 79.

The subjects for our sugar experiments were healthy students (men and women). All were familiar with the technique of venapuncture, both as operators and as subjects. The blood samples were taken from an arm vein by skilled operators, and there was not the slightest ground for suspecting psychic effects on blood sugar in any of the experiments reported here.

## ABSTRACTS OF THE COMMUNICATIONS.

## MINNESOTA BRANCH.

### FIFTH MEETING.

*Minneapolis, Minnesota, May 10, 1922.*

203 (1950)

### The diagnostic value of phosphate metabolism in experimental rickets.

By **J. F. McCLENDON.**

[*From the Laboratory of Physiological Chemistry, University of Minnesota Medical School, Minneapolis, Minn.*]

These results are based on a study of 150 rats, taken from the mothers at the age of three weeks when they weighed about 30 gm. and placed on experimental diets in small separate cages shielded from ultraviolet rays of sunlight. They were not given cod-liver oil or butter fat, but they had sufficient vitamine-A for varying degrees of growth. All of them were x-rayed at the end of this period and turned over to Dr. C. M. Jackson for morphological study. His observations have confirmed my diagnoses.

In order to diagnose rickets in rats the average P metabolism and growth per day were determined (weights being expressed in milligrams) and the following empirical formula was used. Rachitic index = $RI$.

$$RI = \text{increase in body weight} \times 0.0022 - (\text{P retention} - 2).$$

If *RI* is positive the rat has rickets and if negative the rat is normal. For example, if a rat increases in weight 2,000 mg. per day, 2,000 × 0.0022 = 4.4. If its P retention is 4 mg. per day, 4 − 2 = 2 and *RI* = 4.4 − 2 = 2.4, therefore, this rat has rickets. If the P retention is 7 mg. *RI* = — 0.6 and this rat is normal The magnitude of *RI*, if it is positive, denotes the severity of rickets provided the metabolism has been determined over long enough periods and with sufficient accuracy, but the skeleton grows on a "maintenance" diet and this tends to disturb the applicability of the formula. Rats usually retain $\frac{1}{5}$ to $\frac{1}{3}$ of the P of the diet. Since a rat's body contains about 0.5 per cent. P and the normal rate of growth is about 2 grams per day they should retain about 10 mg. P per day or 5 mg. per gm. increase in body weight, and this is found in normal rats of the age studied. As pointed out by McCollum, the rate of growth influences the degree of rickets. I found that during loss of weight, 0.12 per cent. P in the diet prevented rickets whereas during normal growth 0.4 per cent. in the diet may be insufficient to prevent rickets. Every growth promoting substance has a rachitic influence when it promotes growth, therefore, every antirachitic substance that promotes growth may have two opposing actions. In my rachitic rats *RI* varies from 0 to 4 and in my normal rats *RI* varies from − 1 to − 5 due to storage of P in excess of the "minimum" requirement. The use of *RI* in diagnosing rickets has the advantage that it can be done without killing or injuring the animal.

The Ca retention is low in rickets as well as in osteoporosis. If the P intake is lowered the P retention is lowered and the Ca retention is lowered (rickets). If the Ca intake is lowered the Ca retention is lowered but the P retention remains normal (osteoporosis). This was found true for levels of P content of the diet varying from 0.2 per cent. to 0.4 per cent. The Ca content was reduced from 0.8 per cent. in the diet to 0.2 per cent. and the Ca retention became less than $\frac{1}{2}$ the normal. At the end of a month on this diet, the thickness of the bones and the thickness of their walls was reduced. The total volume of the wall substance of the shafts of the long bones was reduced to 53.7 per cent. of the normal.

### 204 (1951)

## The cultivation of Bact. abortus Bang.

By **C. P. FITCH.**

[*From the University of Minnesota, St. Paul, Minn.*]

Many methods have been described for the cultivation of *Bact. abortus* Bang. Following the original work with this organism by Bang and Stribolt in 1897, several investigators have described methods for the cultivation of this germ. Those which are most frequently mentioned, and used are those of Nowak, Holth, Priez, and Fabyan. Recently (1921) Stafseth and Huddleson have described culture media and methods of growing *Bact. abortus* which differ from those already in use. The former recommends a media prepared from liver and spleen. He states that "strains of the abortion bacillus have been isolated more easily by the aid of these media." A glass jar from which the air was partially exhausted by a suction pump, was used in which to grow the cultures. Huddleson emphasizes the importance of an increased carbon dioxide-tension for growing *Bact. abortus* Bang. His conclusions are as follows:

"There is sufficient proof that:

"(1) The growth of *Bact. abortus* is not due to a reduced oxygen tension.

"(2) A carbon-dioxide tension greater than that of the air governs and greatly facilitates the primary growth of *Bact. abortus*.

"(3) An atmosphere containing (by volume) 10 per cent. of $CO_2$ gas appears to produce the earliest and most luxuriant growth of *Bact. abortus*."

Huddleson recommends the use of a generator containing calcium carbonate to which hydrochloride acid is added as a source of the $CO_2$.

We have been working with *Bact. abortus* for many years and have experienced the same difficulty of isolating the organism as described by other authors. All the mentioned methods have been used with more or less success. The abortion germ usually grows with great difficulty in the cultures made from the original infected material as the stomach contents of an aborted fetus. After two or three transfers, the bacilli grow quite readily even under ordinary aërobic conditions.

We have been using the method described by Huddleson, but have used in place of a $CO_2$ generator described by him, commercial $CO_2$ from a tank. This is because too much chlorine was given off with the gas from the Huddleson generator. We also used a serum agar media, the agar being made from lean beef and when used 10 per cent. of naturally sterile horse serum was added to the melted agar, cooled to 50° C., and the tubes are allowed to solidify in a slanting position. The reaction of the agar is adjusted to a $P_H$ of 6.8 to 7.2, which is slightly more alkaline than heretofore recommended.

These culture tubes are heavily seeded with the material to be cultured, and are placed in a Whitall Tatum museum jar to which 10 per cent. $CO_2$ is added. The jar is then sealed and placed in an incubator at 37½° C. After 24 hours' incubation, small pin-point colonies will be observed, and after 48 hours' incubation, well-developed colonies of *Bact. abortus* will be noticed.

Huddleson makes the statement that $CO_2$ accelerates and favors the growth of *Bact. abortus*. We have been able to obtain the same results, using 10 per cent. hydrogen. It would therefore seem that the diminished oxygen tension rather than any specific effect of the $CO_2$, is involved. This last was suggested by Edwards in his review of the article by Huddleson.

205 (1952)

## The effect of phenolsulphonephthalein upon the glomerular circulation in the frog.

By **ARTHUR D. HIRSCHFELDER** and **RAYMOND BIETER**.

[*From the Department of Pharmacology, University of Minnesota, Minneapolis, Minn.*]

Further studies upon the function of the frog's glomeruli observed by the method of A. N. Richards[1] have shown that phenolsulphonephthalein is also excreted through the glomeruli. After the administration of phenolsulphonephthalein the glomerular capsule can be seen to be distended with pink fluid indicating that it is excreted through the glomerulus in an alkaline urine. Rowntree and Geraghty[1, 2] have previously shown that phenolsulphonephthlaein is excreted through the tubules of the kidney but made no observations upon the excretion through the glomeruli. Under direct observation phenolsulphonephthalein can also be seen in the renal tubules, usually as a red dye indicating a deep alkalinity. The tissues immediately surrounding the renal capillaries are in some animals stained a diffuse red by the phenolsulphonephthalein and in some a light yellow, indicating that the tissues are sometimes alkaline and sometimes acid to phenolsulphonephthalein (sometimes more and sometimes less alkaline than $P_H - 7.2$) in spite of the fact that there was in all cases free and fairly rapid circulation visible through the capillaries. This indicates that the reaction of tissue cells may be on the acid side of neutral in spite of the fact that there is no actual asphyxia.

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[1] Richards, A. N., *Am. J. Med. Sc.*, 1922, clxiii, 1.

### 206 (1953)

## A method for the volumetric study of the human hypophysis cerebri with illustrative results.

By **A. T. RASMUSSEN** and **RUTH HERRICK.**

[*From the Department of Anatomy, University of Minnesota Medical School, Minneapolis, Minn.*]

The importance of the ductless glands in modern medicine has created a demand for more accurate data on these structures. Hammar of Upsala has particularly stressed this point. The need for quantitative facts is more urgent in the case of the hypophysis, or pituitary body, because this organ is composed of several parts which are more or less distinct structurally, functionally and embryologically; and because of the present tendency to classify pituitary disorders from the standpoint of hyper- or hyposecretion of each lobe, the time of onset with reference to

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[1] Rowntree, L. G. and Geraghty, J. T., *J. Pharm. and Exper. Therap.*, 1910. i. 579.

[2] Rowntree, L. G. and Geraghty, J. T., *Arch. Int. Med.*, 1912, ix, 284.

adolescence, whether neoplastic or non-neoplastic, etc. The final justification for such a classification of the clinical pictures will depend, at least in a large measure, upon very careful post-mortem analysis of the hypophysis in large series of selected cases. But before pathological material can be properly evaluated it is necessary to establish something of a standard from normal cases, and to determine the range of variability of apparently healthy individuals.

Aside from the gross weight and the length of the three diameters, we have been unable to find on human material anything better than rough qualitative statements and these are greatly at variance with each other. This is particularly true of the relative number and arrangement of the three types of cells in pars anterior and in the amount of colloid. We know of no accurate volumetric data on the size of the various lobes in the hypophysis of man. We have, therefore, set out to determine the relative and absolute weight of the three principal parts of the main body of the gland and of the large colloid masses, together with the relative number and arrangement of the three types of cells in pars anterior of normal adult males between 20 and 60 years of age. For this material we are greatly indebted to the department of pathology, University of Minnesota.

Having made sufficient determinations to establish a comparatively simple routine method applicable to material obtained during regular autopsies, we are summarizing the method that others may profit by our efforts.

## METHOD.

After trying various fixing fluids, we found that none were better than ordinary formalin, 15 to 30 per cent. in strength. If detailed work on the cytoplasmic granules is contemplated, the formalin may be neutralized with magnesium carbonate and the tissue chromated after the preliminary formalin fixation. For all general purposes, chromation is unnecessary. The advantage of formalin is that it not only admits of a sharp differential staining of the cells but produces practically no change in the weight of the hypophysis for several hours and but little modification even after several days. It is therefore possible to get the weight of the fresh organ from formalin material, without the necessity of having delicate balances at the autopsy and without delaying fixation.

To avoid injury to the posterior lobe, it is safer to remove the entire sella turcica with the pituitary *in situ* by pinching off the clinoid processes and plunging the entire mass into formalin for later removal of the hypophysis. As soon as possible the excess dural sheath is removed and the infundibular stalk cut off close to the main body of the gland. The organ is again placed in formalin a few moments to moisten the outer surface which becomes somewhat dry as a result of the handling. After blotting off excess formalin the organ is weighed and then fixed three or four days longer in fresh formalin. Without washing in water, the tissue is dehydrated as usual, cleared in xylol and embedded in hard paraffin (60° C.), reducing the duration in the paraffin both to four or five hours by changing the paraffin at least three times.

The organ is cut horizontally, *i.e.*, through its greatest diameter, and not sagittally as in the prevailing method. If one is to depend upon a limited number of sections for an estimate of the condition of the gland, the view obtained in the horizontal plane is by far the best. Mid-sagittal sections are particularly atypical of pars anterior for this region is frequently very poor in eosinophiles. Sections are cut 10 $\mu$ till one fourth through the block when an even number (about 10) of 5 $\mu$ sections are cut for cytological work and differential cell counting. Cutting at 10 $\mu$ is resumed until the middle of the block is reached and again till three-fourths through when similarly a few 5-$\mu$ sections are obtained. These division points may be determined near enough by marking the end of the paraffin block after it is trimmed for sectioning.

The whole series is marked off into groups of twenty sections (two 5-$\mu$ sections counting as one) and from the middle of each group a 10-$\mu$ section is taken. These (usually 30 to 40 in number) can be mounted on three or four slides and stained with Mallory's connective tissue stain (acid fuchsin-aniline blue-orange G) as regularly done in staining connective tissue. Another similar set is taken from points midway between the other series and stained with hematoxylin and eosin in order to have two series which together will constitute a more complete set consisting of every tenth section and also to have a better nuclear stain. Every section of one of these series only (as a rule) is projected at a magnification of twenty diameters upon "American Linen Record" paper (sheets 23 × 36 inches, 72 lbs. per ream) which runs very uniformly at .012 gram per sq. cm. Every sheet needs checking up, however. This is done by cutting out from each corner a square 5 cm. each way and weighing the four squares together. If a heavy sheet is balanced with a light sheet (several sheets being necessary for each determination) the weight per sq. cm. can be kept sufficiently constant. The capsule (including the connective tissue extending in between the lobes), pars anterior, connective tissue trabeculæ and large colloid masses in pars anterior, parenchyma of pars intermedia, colloid in pars intermedia, and pars nervosa are outlined with a hard sharp pencil or with ink. Where the colloid and parenchyma of pars intermedia are very irregularly distributed, it is safer to use both series, thus reducing the error greatly.

These areas are cut out with scissors, using a fine manicuring scissors for cutting out the smaller areas and areas rounded by many sharp turns. The paper representing any particular part is weighed. The percentage that this weight is of the weight of the paper of all parts together constitutes the percentage of the whole organ represented by that part, if the shrinkage is the same for all parts in any particular case.

To determine the shrinkage of the two lobes, they were separated from each other in three cases and each lobe weighed before fixation. The members of each pair were kept together through the entire process of fixation, embedding, etc. From serial sections, as explained above, the final volume was obtained by dividing the total paper weight of a lobe by the weight of one sq. cm. of paper, then dividing the results by the actual magnification (magnification in diameter squared) and finally multiplying by 200 $\mu$ (20 × 10 $\mu$) reduced to cm. (.02 cm.). The original volume (before fixation) was determined by dividing the weight by the specific gravity.

The results are tabulated in Table I, from which it is seen that there is sufficiently close agreement between the shrinkage of the two parts in each case that it may be considered the same despite the great difference in structure.

TABLE I.

SHRINKAGE PRODUCED BY THE TECHNIQUE WHEN THE POSTERIOR AND ANTERIOR LOBES WERE SEPARATED FROM EACH OTHER.

| Autopsy Number. | 20–446. | | 20–450. | | 22–140. | |
|---|---|---|---|---|---|---|
| Lobe | Anterior. | Posterior. | Anterior. | Posterior. | Anterior. | Posterior. |
| Original volume, c.c. | .3632 | .1415 | .3113 | .1057 | .2547 | .1113 |
| Final volume, c.c. | .2430 | .0950 | .1928 | .0666 | .1540 | .0691 |
| Shrinkage, c.c. | .1202 | .0465 | .1185 | .0391 | .1007 | .0422 |
| Per cent. of shrinkage | 33.1 | 32.9 | 38.1 | 37.0 | 39.5 | 38.0 |

This is a method which has been used extensively for similar purposes in this and other laboratories and especially by Godlewski, Hammar and Jackson, and the senior author has used it on the hypophysis of the woodchuck. Whether any time would be saved by measuring the areas with a planimeter has not yet been determined on this material. Dr. C. M. Jackson, of this laboratory, after comparing the two methods, favored the cutting-out process. The planimeter cannot increase the accuracy providing one has dexterity with the scissors.

For a differential count of the types of cells in pars anterior, a 5-$\mu$ section from each of the three planes mentioned is stained with Mallory's connective tissue stain, going rapidly through the alcohols following the stain. No better differential stain could be wished than this will give on material only a few hours post mortem. These three sections are systematically explored with an oil-immersion lens by means of a mechanical stage, and the cells of each type in each fifth field of each fifth row (as shown in Fig. 1) are counted and the percentage of the total calculated.

## RESULTS.

In Table II are recorded some selected cases to illustrate what the method yields in four normal males with hypophyses covering the usual range in weight met with, one female with a rather large hypophysis and one case of non-neoplastic post-adolescent hypopituitarism of both lobes. The small normal male hypophysis (Nos. 21–66) is seen to have an especially small pars nervosa (posterior lobe). The great size of the female hypophysis is due to a large pars anterior. The hypopituitary case has a hypophysis very similar in volumetric relations to the female. The absence of data on the parenchyma of pars intermedia of the female hypophysis is due to the fact that the cells of this portion in this particular case were very few and the tissue not fresh enough when fixed to be well differentiated by the stain. This will have to be expected in a few cases since pars intermedia constitutes such a small irregular part of the entire gland—a fact of some consequence in view of Bab's theory that the polyurea in hypopituitarism is due to hyposecretion of the pars intermedia and because in some of the lower forms this part of the hypophysis is distinctly connected with pigmentation. The greatest error will always be in connection with the juxtaneural portion; but the error can be decreased by placing this part of the gland under higher magnification if that should be deemed necessary.

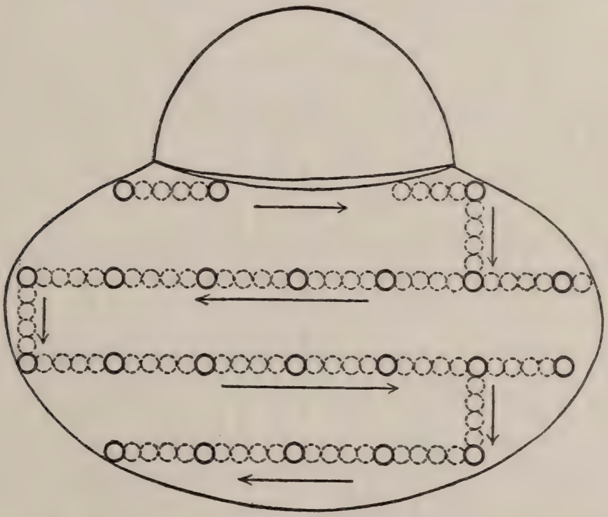

FIG. 1. Diagram showing how the field is explored in making the differential count of cells in pars anterior. The solid circles represent the fields which are actually used.

In Table III is shown a typical cell count as obtained by the method here outlined.

## TABLE II.

### WEIGHT AND PERCENTAGE OF THE VARIOUS PARTS OF THE HUMAN HYPOPHYSIS.

| Cases. | Autopsy Number.. | 20–323. | | 20–380. | | 21–68. | | 21–66. | | 21–14. | | 22–81. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Age........ | 36 yr. | | 37 yr. | | 50 yr. | | 58 yr. | | 53 yr. | | 59 yr. | |
| | Sex........ | Male. | | Male. | | Male. | | Male. | | Female. | | Male.[1] | |
| Weight of Whole Gland (grams)................ | | .530 | | .660 | | .585 | | .420 | | .830 | | .620 | |
| | | grams. | % | grams. | % | grams. | % | grams. | % | grams. | % | grams. | % |
| Pars Anterior (Distalis) | Trabeculae . | .0043 | .84 | .0032 | .49 | .0099 | 1.70 | .0072 | 1.71 | .0159 | 1.92 | .0022 | .34 |
| | Parenchyma | .3832 | 72.30 | .4501 | 68.20 | .3743 | 63.99 | .2859 | 68.08 | .6339 | 76.37 | .4678 | 75.45 |
| | Total...... | .3875 | 73.14 | .4533 | 68.69 | .3843 | 65.69 | .2931 | 69.79 | .6498 | 78.29 | .4700 | 75.79 |
| Pars Nervosa (Proc. Infund.) or Posterior....... | | .1120 | 21.13 | .1538 | 23.30 | .1319 | 22.54 | .0722 | 17.20 | .1248 | 15.04 | .0939 | 15.15 |
| Pars Intermedia (Juxtaneuralis) | Colloid..... | .0046 | .86 | .0022 | .33 | .0055 | .94 | .0036 | .86 | .0093 | 1.12 | .0101 | 1.63 |
| | Parenchyma | .0022 | .42 | .0110 | 1.67 | .0104 | 1.77 | .0040 | .95 | ? | ? | .0074 | 1.20 |
| | Total...... | .0068 | 1.28 | .0132 | 2.00 | .0159 | 2.71 | .0076 | 1.81 | ? | ? | .0175 | 2.83 |
| Capsule................................ | | .0234 | 4.42 | .0389 | 5.90 | .0259 | 9.05 | .0471 | 11.21 | .0460 | 5.54 | .0386 | 6.22 |

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[1] A typical specimen of so-called non-neoplastic post-adolescent hypopituitarism of both lobes (diagnosis by Dr. Wm. O'Brien). 189 cm. long. Weight 330 lbs. 3 hrs. post mortem.

TABLE III.

DIFFERENTIAL COUNT OF THE CELLS IN PARS ANTERIOR OF HYPOPHYSIS FROM A 63-YR.-OLD WOMAN (AUTOPSY NUMBER 21-190—3 HR. POST MORTEM).

Total fields from three different levels 150. Sections 5 $\mu$ thick. Formalin fixation. Mallory's C.T. Stain.

| Cell Types. | All Types Together. | Chromophobes. | Acidophiles. | Basophiles. |
|---|---|---|---|---|
| Number of cells counted.. | 25,658 | 14,776 | 8,510 | 2,372 |
| Percentage.............. | 100 | 57.6 | 33.2 | 9.2 |

## ABSTRACTS OF THE COMMUNICATIONS.

## WESTERN NEW YORK BRANCH.

*Ithaca, New York, May 20, 1922.*

### 207 (1954)

### The effect of thyroidectomy on the intelligence of sheep.

By **H. S. LIDDELL** (by invitation).

[*From the Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

The procedure followed in attempting to determine the effect of thyroidectomy on the intelligence of sheep has already been described.[1] Four pairs of twin female lambs from three to four weeks old were caused to learn a labyrinth with a single cul de sac and the twin of each pair making the better record was thyroidectomized by Dr. Simpson. Two months later the operated lambs had become typical cretins.

One hundred two days following thyroidectomy one of the cretins, already definitely lethargic, and her normal twin began relearning the labyrinth. The position of the cul de sac was then reversed and the labyrinth was again learned. Learning was taken to be complete at the end of three trials without error; an error being counted whenever the animal entered the cul de sac or turned back along the true path. The number of steps taken by the animal and the time in seconds were recorded for each trial. The results were as follows:

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[1] Liddell H. S., PROC. SOC. EXPER. BIOL. AND MED., 1922, xix, 343.

RELEARNING THE SIMPLE LABYRINTH.

| | Total No. of Trials. | Total No. of Errors. | Total Time. |
|---|---|---|---|
| Normal Lamb..... | 4 | 4 | 472 sec. |
| Cretin Lamb...... | 3 | 0 | 173 sec. |

LEARNING THE LABYRINTH WITH THE POSITION OF THE CUL DE SAC REVERSED.

| | Total No. of Trials. | Total No. of Errors. | Total No. of Steps. | Total Time. |
|---|---|---|---|---|
| Normal Lamb..... | 6 | 27 | 3176 | 1226 sec. |
| Cretin Lamb...... | 5 | 34 | 6016 | 4821 sec. |

One hundred forty-seven days following extirpation of the thyroids another cretin, markedly lethargic, was tested. She was given three trials but was unable to escape from the labyrinth which she had learned before being thyroidectomized. The results of these tests were:

RELEARNING THE SIMPLE LABYRINTH.

| | | No. of Errors. | No. of Steps. | Time. |
|---|---|---|---|---|
| Cretin Lamb ... | 1st trial | 46 | 3548 | Removed in 2 hrs. |
| | 2d " | 16 | 1570 | " " 2 " |
| | 3d " | 10 | 1193 | " " 1½ " |

| | Total No. of Trials. | Total Errors. | Total Steps. | Total Time. |
|---|---|---|---|---|
| Normal Lamb .. | 6 | 8 | 3116 | 963 sec. |

The other two cretins did not become noticeably lethargic, although they were dwarfed in stature as compared with their twins. Tests were begun on one of these lambs and her normal twin two hundred six days following thyroidectomy. The results were:

RELEARNING THE SIMPLE LABYRINTH.

| | Total No. of Trials. | Total No. of Errors. | Total No. of Steps. | Total Time. |
|---|---|---|---|---|
| Normal Lamb..... | 4 | 4 | 2452 | 552 sec. |
| Cretin Lamb...... | 4 | 3 | 3548 | 557 sec. |

LEARNING THE LABYRINTH WITH THE POSITION OF THE CUL DE SAC REVERSED.

| | Total No. of Trials. | Total No. of Errors. | Total No. of Steps. | Total Time. |
|---|---|---|---|---|
| Normal Lamb..... | 7 | 4 | 3254 | 801 sec. |
| Cretin Lamb...... | 4 | 12 | 4710 | 2319 sec. |

Tests on the remaining pair were begun two hundred seven days after the operation with the following results:

RELEARNING THE SIMPLE LABYRINTH.

| | Total No. of Trials. | Total No. of Errors. | Total No. of Steps. | Total. Time. |
|---|---|---|---|---|
| Normal Lamb..... | 5 | 11 | 2808 | 911 sec. |
| Cretin Lamb...... | 4 | 5 | 3312 | 450 sec. |

LEARNING THE LABYRINTH WITH THE POSITION OF THE CUL DE SAC REVERSED.

| | Total No. of Trials. | Total No. of Errors. | Total No. of Steps. | Total Time. |
|---|---|---|---|---|
| Normal Lamb..... | 8 | 5 | 4804 | 828 sec. |
| Cretin Lamb...... | 4 | 9 | 3553 | 1375 sec. |

A more difficult problem was then presented to this pair. The position of the cul de sac was alternated during four successive trials per day and twelve trials without error were required for complete learning. Three hundred thirteen days after thyroidectomy the cretin completed the problem in two hundred twenty trials while the normal lamb required but one hundred fifty-six trials.

The expense of this investigation was defrayed by grants from the Heckscher and Sage Research Foundations.

208 (1955)

## The characteristic electrocardiogram of the cretin sheep.

By **STANLEY ROSS BURLAGE** (by invitation).

[*From the Electrocardiographic Laboratory, Department of Physiology, Cornell Medical College, Ithaca, N. Y.*]

In November, 1921, the writer made routine electrocardiograms of Dr. Sutherland Simpson's group of experimental cretin lambs and the control normal lambs.

The three leads employed were the same as those used in the usual human electrocardiograms. Soft sheet lead electrodes were bound on the clipped upper leg of the sheep by means of cloth strips wet with saturated salt solution. The lambs stood in the normal position.

A brief review of Dr. Simpson's treatment of the sheep is essential in interpreting the results.

In Group I there were 4 pairs of twin lambs. One of each pair had been operated upon when from 6 to 8 weeks old and both lobes of the thyroid removed. The other was left unoperated as a control.

Two of the operated lambs began to receive thyroxin subcutaneously, beginning 17 to 21 weeks after the operation and lasting until the electrocardiograms were taken. The period of treatment was approximately one month.

The electrocardiograms of the cretins which had received no treatment definitely differed from the normal. In these former the *P* and *Q* waves were either absent or just discernable in all derivations. The *R* and *T* waves were very small; the latter being negative in all cases. This is the normal direction for this final deflection in the sheep electrocardiogram.

In the case of the cretins which had received thyroxin the *P* waves were nearly normal in height in all derivations; there were definite *Q* waves; the *R* waves were increased in height and the *T* waves were larger and positive in direction.

In Group II there were 5 pairs of twin lambs. Of 4 pairs of these twins, one of each pair was completely thyroidectomized when 4 to 6 weeks old. 30 weeks later each of these operated lambs received treatment for 15 weeks and electrocardiograms were taken 28 weeks after treatment was stopped.

The cretin treated with NaI in the feed, and the one treated with thyroxin subcutaneously, showed the typical cretin electrocardiograms. Of the two lambs given thyroid extract in the feed, one showed an electrocardiogram slightly more normal than that of the untreated cretin, and the other presented an almost normal electrocardiogram.

One of the 5th pair of twins was completely thyroidectomized when 23 weeks old. Subcutaneous treatment with thyroxin was begun 13 weeks later and continued for 15 weeks. 28 weeks later the electrocardiograms were taken. They showed a distinct advance toward the normal from the cretin type.

The expenses incurred in these experiments were defrayed by a grant from the Heckscher and Sage Research funds, made to Dr. Simpson.

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209 (1956)

## Preliminary studies of "posterior paralysis" in swine.

By L. A. MAYNARD.

[*From the Department of Animal Husbandry, Cornell University, Ithaca, New York.*]

A study is in progress of the frequently occurring trouble in swine, commonly referred to as "leg weakness" or "posterior paralysis." Some preliminary observations are here described.

In connection with another experiment, a group of 4 pigs, each about 12 weeks of age, were fed in dry lot for 90 days on a ration consisting of 1 part of yellow hominy feed and 1.4 parts of pasteurized skim milk, together with a mineral mixture made up of charcoal, ground limestone and common salt.

At or near the end of the 90-day period, 3 of the 4 pigs developed symptoms of trouble. A stiffness of the hind legs first became evident. The skin became dry, scaly and covered with large, livid spots. The stiffness increased, accompanied by pain. A swelling of the knee joints was noted in one individual. Finally, paralysis developed in the hind legs of 2 of the animals, they being able neither to rise nor to stand. A rapid loss in weight set in at this stage.

At the close of the 90-day period, the writer changed the diet for 2 of the pigs. One of them, number 122, was unable to rise or stand, was obviously in pain and was losing rapidly in weight; the other, No. 107, was markedly stiff behind but was still able to walk. To the hominy-skim milk ration, was added 4 ozs. of carrots per animal daily. The mineral mixture was replaced by precipitated bone meal, precipitated calcium carbonate and salt.

After 10 days, a decrease in the stiffness and in the skin troubles was noted in pig 107, and rapid growth was resumed. At the end of 5 weeks the stiffness had disappeared and the skin was nearly normal. During the last three weeks the pig gained over 2 lbs. a day, compared to 1 lb. at the close of the 90-day period. Pig 122 showed the first definite evidence of recovery after 3 weeks, when it was able to stand momentarily if placed on its feet. It could get up by itself and take several steps at 4 weeks. At 7 weeks, lost weight had been recovered, the skin was nearly normal, the pain appeared to be entirely gone, and the animal was able to get around rather freely, though stiffness was still present.

### 210 (1957)

## The pathological tissue changes resulting from feeding cottonseed meal.

By **S. A. GOLDBERG** and **L. A. MAYNARD.**

[*From the Department of Comparative Pathology and the Department of Animal Husbandry, Cornell University, Ithaca, N. Y.*]

Twelve pigs were fed a ration of cornmeal 60 per cent., wheat middlings 10 per cent., molasses 5 per cent. and cottonseed meal 10 per cent. which was gradually increased to 25 per cent. They also received a mineral mixture of salt and lime. Six of these died from 8 to 13 weeks after the experiment was begun, the rest were at that time changed to a ration free from cottonseed meal and are still alive.

The most constant tissue changes were ascites, hydrothorax, hydropericardium, a distension of the sheath, anasarca, œdema of the perirenal fat and congestion of the thyroid glands with a marked diminution of colloid material. In those that died first, there were marked subpleural and interlobular pulmonary œdema. The œdematous liquid was slightly reddened and gelatinized on standing.

A Holstein cow has been fed cottonseed meal for 10 weeks beginning with 6 quarts and gradually increased to 10 quarts daily comprising the entire grain ration, with hay for roughage.

She does not show any ill effects as yet. Two adults, a boy 5 years old, and two dogs, fed milk of this cow the first week of the experiment, developed diarrhea. Two dogs fed the milk the second week did not show any ill effects. A two-day-old calf began sucking this cow 15 days after the experiment was started. He gained weight steadily and showed no apparent ill effects. The calf was killed by bleeding from carotid at the age of 5 weeks. Autopsy showed œdema of the perirenal fat and of the omentum. In the peritoneal cavity, there was about 600 c.c. reddish liquid that gelatinized on standing, similarly to the ascitic liquid found in the pigs. All the other organs appeared normal.

### 211 (1958)

## A comparison of the alkaline tide in urine with the results of fractional gastric analysis.

By ROGER S. HUBBARD and SAMUEL A. MUNFORD.

[*From the Clifton Springs Sanitarium, New York.*]

The fact that the acidity of the urine decreases after meals is well known, and this decrease has been commonly attributed to the secretion of hydrochloric acid by the stomach during the process of digestion, but there are comparatively few experiments recorded in which direct comparison between urine studies and analyses of the acid content of the stomach by the newer methods have been made. In the series of cases presented determinations of hydrogen-ion concentration, titratable acidity, ammonia, and in some instances, total nitrogen, were made on urine collected at two-hour intervals through the day from patients who had been studied for hyperacidity or anacidity of the stomach by the usual method of fractional gastric analysis. Those cases which showed free hydrochloric acid in the stomach showed the "alkaline tide" in the morning, and usually in the afternoon as well, while the cases which did not show hydrochloric acid, with one possible exception, did not show the tide.

The depth and duration of the alkaline tide did not correspond with the relative amounts of hydrochloric acid found in the gastric contents, or with the form of the acid curve. Determination of the alkaline tide furnished a method for checking the findings in the gastric juice, and has been found useful in establishing a possible anacidity in cases where satisfactory gastric analyses could not be obtained.

### 212 (1959)

## The blood chemistry of thyroidectomized sheep.

By **AARON BODANSKY** (by invitation).

[*From the Biochemical Laboratory, Department of Physiology and Biochemistry, Cornell University Medical College, Ithaca, N. Y.*]

A study of the blood chemistry of Dr. Sutherland Simpson's experimental flock has been undertaken as a part of a larger study of the metabolism of thyroidectomized sheep.

The flock consists of thyroidectomized individuals and normal controls. The thyroidectomized sheep were operated on at different ages, and to some of them were later administered thyroxin, thyroid gland or sodium iodide in the course of other experimental work. These administrations were discontinued about 10 months before the beginning of this series of analyses.

Some of the animals were withdrawn from the author's use for shorter or longer periods, and others were used simultaneously for other experimental work involving increased exercise. In the preliminary tests no attempt was made to control the diet of the animals in any manner. Later the uniform practice was adopted of taking the blood after a night of enforced rest in the sheep pens before the sheep were given their morning meal. Greater uniformity of results was then obtained and it became possible to observe certain broad distinctions between the normal and operated groups.

The blood was drawn from the jugular vein directly into tubes containing finely powdered potassium oxalate, which were kept in ice water until used for analyses. Folin and Wu's procedure was employed.

At this time enough determinations are available only for sugar and N. P. N. Sugar was found to range between 0.06 and 0.07

grams per 100 c.c. of blood for the normal animals and between 0.04 and 0.05 for the thyroidectomized ones. (A sharper division was observed when the blood was obtained several hours after the morning meal. The normal animals showed 0.1 – 0.12 grams per 100 c.c.; the thyroidectomized group showed 0.06 — 0.07 grams per 100 c.c.)

The values observed for N. P. N. were as follows: For normal sheep, 40–42 mgms. per 100 c.c., for thyroidectomized sheep 28–32 mgms.

Other data are incomplete, and will be reported later in connection with a new series of analyses to be undertaken on animals under better experimental control.

The work was carried out under grants from the Sage and Heckscher research funds made to Dr. Sutherland Simpson.

### 213 (1960)

## Basal metabolism of premature and undersized infants.

By JOHN R. MURLIN and ELIZABETH MARSH.

[*From the Physiological Laboratory, University of Rochester, and from the Obstetrical Division of Highland Hospital, Rochester, N. Y.*]

The basal metabolism of infants must be obtained during sleep and infants naturally sleep best when recently fed. Following the procedure employed by Bailey and Murlin[1] the infants of the present series were studied under those conditions while exposed in the respiration incubator[2] to an environing temperature of 27 to 29° C.

Studies on two premature infants were reported by Rubner and Langstein[3] in 1915 and a preliminary report of several cases has been made recently by Talbot.[4] The former authors found the metabolism of a 2 months' premature infant on the 27th day after birth to be 973 calories per sq. meter per 24 hours (Meeh-

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[1] Bailey and Murlin, *Journ. of Obstet.*, 1915, lxxi, No. 3.

[2] Murlin, J. R., *Amer. Journ. Dis. of Children*, 1915, ix, 42–58.

[3] Rubner and Langstein, *Archiv. f. Physiologie*, 1915, p. 39.

[4] Talbot, F. B., PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 309.

METABOLISM OF PREMATURE AND UNDERSIZED INFANTS.

| Name. | Sex. | Birth Weight. | Maturity. | Date. | Age (Days). | $CO_2$ (Liters). | $O_2$ (Liters). | R.Q. | Calories per Sq. M. | Calories per Kgm. |
|---|---|---|---|---|---|---|---|---|---|---|
| Levine Twin I | F. | 2213 gms. | Full | 1-31-22 | 6 | 0.5832 | 0.7173 | 0.81 | 26.15 | 2.06 |
| | | | | | | 0.5087 | 0.6578 | 0.77 | 23.74 | 1.87 |
| | | | | 2- 2-22 | 8 | 0.6528 | 0.7767 | 0.84 | 25.15 | 1.95 |
| | | | | | | 0.6639 | 0.9436 | 0.70 | 29.14 | 2.26 |
| Levine Twin II | F. | 2156 gms. | Full | 1-31-22 | 6 | 0.5455 | 0.6319 | 0.87 | 25.70 | 2.01 |
| | | | | | | 0.4708 | 0.5059 | 0.93 | 18.79 | 1.47 |
| | | | | 2- 2-22 | 8 | 0.7423 | 0.9349 | 0.79 | 26.83 | 2.07 |
| | | | | | | 0.7261 | 0.9349 | 0.78 | 26.60 | 2.05 |
| Jex | F. | 2770 gms. | 8½ mo. | 4-30-21 | 5 | 0.8027 | 0.8635 | 0.93 | 27.57 | 2.19 |
| | | | | | | 0.5900 | 0.7194 | 0.82 | 26.81 | 2.13 |
| Tecklow[1] | M. | 2326 gms. | 8½ mo. | 3- 6-22 | 1 | 0.6297 | 0.9020 | 0.70 | 27.57 | 2.12 |
| Graham[2] | M. | 2383 gms. | 8½ mo. | 4- 5-22 | 23 | 0.6606 | 0.7998 | 0.83 | 24.06 | 2.09 |
| Schlinger | F. | 1589 gms. | 8¼ mo. | 3-13-22 | 3 | 0.5251 | 0.7453 | 0.70 | 24.95 | 2.21 |
| | | | | 3-17-22 | 7 | 0.5707 | 0.8719 | 0.65 | 23.04 | 2.04 |
| | | | | | | 0.4175 | 0.4995 | 0.84 | | |
| | | | | 3-17-22 | 7 | 0.4510 | 0.5997 | 0.75 | 23.42 | 2.07 |
| Payne[3] | M. | 1872 gms. | 7½ mo. | 3- 1-22 | 4 | 0.3786 | 0.5102 | 0.74 | 25.84 | 2.13 |
| | | | | | | 0.3487 | 0.4283 | 0.81 | 22.73 | 1.87 |
| | | | | 3- 1-22 | 4 | 0.3815 | 0.3982 | 0.96 | 17.70 | 1.46 |
| | | | | 3- 3-22 | 6 | 0 3745 | 0.4527 | 0.83 | 20.78 | 1.72 |
| | | | | | | 0.2649 | 0.3933 | 0.67 | | |
| | | | | 3- 3-22 | 6 | 0.4712 | 0.6389 | 0.74 | 20.68 | 1.71 |
| | | | | | | 0.6344 | 0.5584 | 1.14 | | |
| Molyneux Twin I | M. | 2213 gms. | 7 mo. | 2- 6-22 | 10 | 0.6868 | 0.7963 | 0.86 | 26.19 | 2.06 |
| | | | | 2- 8-22 | 12 | 0.6802 | 0.9083 | 0.75 | 29.85 | 2.32 |
| Molyneux Twin II | M. | 2043 gms. | 7 mo. | 2- 6-22 | 10 | 0.9040 | 1.009 | 0.90 | 28.46 | 2.28 |
| Brown[3] | F. | 1248 gms. | 7 mo. | 3-14-21 | 9 | 0.5826 | 0.6550 | 0.89 | 19.50 | 1.95 |

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[1] Cæsarean birth; died.

[2] Case of sclerema monotarum; died.

[3] Died.

Rubner formula), or 40 calories per sq. meter per hour. This metabolism includes the normal muscular activity (crying) of the infant for the day. Bailey and Murlin found the basal as an average of 13 separate determinations on six infants born at term, within the age of 12 days, to be 23.7 calories per sq. meter per hour (Meeh-Rubner formula), or 27.3 calories for the Lissauer formula. Benedict and Talbot[1] found 25.5 calories per sq. meter (Lissauer) an hour as the average for 94 newborn infants at an average of two days.

The present report includes results on ten newborn infants all of whom were under 5 lbs. 5 oz. at birth. Two (twins) were born at term, three at 8½ months, one at 7½ months and three, two of whom were twins, at 7 months. The average heat production (basal) was 24.63 calories per sq. meter (Lissauer) per hour. The table epitomizes the results. Four of the ten, one a Cæsarean, one a case of sclerema monatorum and two from prematurity only subsequently died. All the others at last reports were growing.

The respiratory quotients found range from 0.70 to 0.93 according to state of nutrition, *i.e.*, time from feeding.

Of the four who died the basal metabolism of all except the Cæsarean case, at the time of observation were distinctly below the average for infants born at term (see above). The average of the remaining six was 25.8 calories per sq. meter per hour (Lissauer).

Since basal metabolism is the best single measure of vitality yet found it is probable that this test will prove to be an important means of prognosis in prematurity.

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[1] Benedict and Talbot, Carnegie Inst. of Wash. Publ. No. 233, 1915.

# RECAPITULATION OF THE NAMES OF THE AUTHORS AND OF THE TITLES OF THE COMMUNICATIONS.

## VOLUME XIX.

**Avery, O. T.**

1801. [with **Hugh J. Morgan.**] The effect of the accessory substances of plant tissue upon growth of bacteria.

**Abramson, Harold A.**

1802. [with **Samuel H. Gray.**] The diffusion of sodium chloride through a "lecithin" collodion membrane.

**Anderson, J. F.**

1852. A note on the preparation of anti-colon streptococcus serum.

**Ascham, Leah.**

1809. See **Smith, Arthur H.**

**Auster, Lionel S.**

1803. [with **Burrill B. Crohn.**] Notes on studies in the physiology of the gall bladder.

**Bagg, Halsey J.**

1933. See **Edwards, D. J.**

**Bailey, C. V.**

1896. An appliance to be used with a gasometer for recording the volume of each expiration.

**Barnett, Marion.**

1824. See **Zucker, T. F.**

**Barr, David P.**

1828. The acid-base equilibrium of the blood following vigorous muscular exercise.

**Bauguess, Harry.**

1778. See **McClendon, J. F.**

**Bell, Marion.**

1941. [with **L. B. Mendel.**] The distribution of vitamine-B in the wheat kernel.

**Bentz, C. A.**

1856. See **Lathrop, Carl O.**

**Bercowitz, Z.**

1846. Preliminary report on the effects of vagus stimulation on the stomach of a dog and the influence of asphyxia on these effects.

**Bergeim, Olaf.**

1759. See **Smith, Clarence A.**

**Bieter, Raymond N.**

1916. [with **Arthur D. Hirschfelder.**] Effect of sodium benzoate, sodium hippurate and other drugs upon the glomerular circulation in the frog.

1952. See **Hirschfelder, A. D.**

**Bodansky, Aaron.**

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1912. See **Mellon, Ralph R.**

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1793. Effect of experimentally induced changes in consistency on protoplasmic movement.

1894. [with **Hiroshe Ohshima.**] Merogony experiments on sea-urchin eggs.

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1835. See **Krumbhaar, E. B.**
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1927. See **Kopeloff, Nicholas.**
**Cheplin, Harry A.**
1785. See **Rettger, L. F.**
**Churchman, John W.**
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1787. Some phases of the disinfection theory.
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1827. [with **R. L. Levy.**] Experiments with quinidine on conduction and on the refractory period in the dog's heart.
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1902. See **Crozier, W. J.**
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1829. See **Bowen, R. J.**
**Cowgill, George R.**
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1871. Parenteral administration of products containing vitamine-B.—Mammalian experiments.
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1803. See **Auster, Lionel S.**
**Croll, Hilda M.**
1790. See **Kast, Ludwig.**
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1893. See **Sherwin, Carl P.**
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**DeEds, Floyd.**
1884. See **Hanzlik, P. J.**
**De Kruif, Paul H.**
1764. Dissociation of microbic species. II. Mutation in pure line strains of the bacillus of rabbit septicemia.
1765. III. Differentiation of microbes D and G by acid agglutination.
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1767. V. Further considerations in regard to the virulence of microbes D and G.
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1913. See **Schmidt, Carl L. A.**
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1798. [with **Georgina S. Burke.**] Botulism—a method for determining the thermal death time of the spores of bacillus botulinus.
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1857. [with **C. B. Higley.**] An intramuscular method of digitalis assay.
**Draper, John W.**
1853. The use of colon-streptococcus anti-serum as a pre-operation measure.
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**Dubin, Harry E.**
1754. See **Funk, Casimir.**
1796. See **Ringer, A. I.**

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1890. A ten-year-old strain of fibroblasts.
**Eddy, Walter H.**
1821. [with **E. Shelow** and **R. A. Pease.**] The effect of cooking upon the antiscorbutic vitamine in cabbage.
**Edson, Philip.**
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1772. [with **Margaret S. Melcher.**] A modification of the DuBois height-weight formula for surface areas of newborn infants.

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1817. The velocity of development of the demarcation current in the frog's sartorius.

**Falk, I. S.**

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1924. [with **Julia A. W. Hewitt.**] The Hecht-Weinberg-Gradwohl reaction in syphilis.

1925. [with **Lucile Robey.**] Pneumococcus grouping on 1000 cases.

1926. See **Hewitt, Julia A. W.**

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1839. [with **Casimir Funk.**] The vitamine requirements of certain yeasts and bacteria.

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1806. Collodion sacs for aërobic and anaërobic bacterial cultivation.

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1859. [with **F. H. Scott.**] The control of respiration.

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1773. [with **Charles S. Capp** and **Frederick S. Foote.**] On the relation of blood-volume to the nutrition of the tissues. I. The effects of hemorrhage and intravenous injection of gum-saline on the response to the administration of a mixture of carbon dioxide and room air, and of room air alone.

1774. II. The effects of hemorrhage and subsequent injections of gum-saline upon the volume flow through striated muscle of the dog.

1775. III. The effects of hemorrhage and subsequent injection of gum-saline upon the response of sartorius muscle of the dog to rapid electrical stimulation.

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1842. See **Brown, S. A.**

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1917. See **Mann, Frank C.**

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1861. [with **W. F. Hoffman.**] Evidence of a structure in gelatine gels.

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1919. See **Newton, Robert.**

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1844. See **Friedman, G. A.**

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1779. Rapid determination of surface tension.

**Gray, S. H.**

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1826. [with **V. Kneeland Franz.**] Observations on the inorganic phosphate of blood in experimental rickets of rats.

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1769. [with **Florence Finnerud.**] The production of tyrosine by a putrefactive anaërobe.

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1761. See **Moloney, P. J.**

1847. See **Moloney, P. J.**

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1837. [with **Mary McIntyre** and **Elizabeth Presho.**] Experimental plumbism; the therapeutic efficiency of some agents and comparative toxicity of other metals.

1883. [with **Howard P. Karsner.**] Further observations on anaphylactoid phenomena from different agents including histamine.

1884. [with **Floyd DeEds** and **Elizabeth Presho.**] Urinary excretion of salicyl after the administration of salicylate and salicyl esters.

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1868. The relation of the diffusion constant to mountain sickness.

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1751. [with **Soma Weiss.**] Further observations on the seat of the emetic action of the digitalis bodies.

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1924. See **Famulener, L. W.**

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1782. [with **L. J. Pankow.**] Does the introduction of an ethoxy group into aromatic compounds increase their bactericidal action upon the pneumococcus and the gonococcus?

1818. [with **Herman H. Jensen.**] The pharmacological action of some ethers and esters of saligenin.

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**Horning, Benjamin.**

1867. See **Torrey, H. B.**

**Honeywell, H. E.**

1931. [with **Oscar Riddle.**] Increase blood sugar coincident with ovulation in pigeons.

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1910. The existence of an attracting stimulus in the development of the central nervous system.

**Hotchkiss, Margaret.**

1889. See **Winslow, C.-E. A.**

**Howe, Paul E.**

1903. See **Orcutt, Marion L.**

**Hubbard, Roger S.**

1795. [with **Floyd R. Wright.**] Studies on the acetonuria produced by diets high in fat.

1877. [with **David C. Wilson.**] An experiment on the absorption of glucose given by rectum.

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**Johnson, B. W.**

1823. See **Little, C. C.**

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1810. See **Kahn, R. L.**

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1830. A simple precipitation reaction for syphilis.

1831. A simple quantitative precipitation reaction for syphilis—micro procedure.

1878. Effect of dilution on the precipitation reaction for syphilis proposed by author.

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1790. [with **Hilda M. Croll.**] Observations on the excretion of sugar in the urine in health and disease.

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1811. [with **Philip J. Rosenbloom.**] The prevention and control of parathyroid tetany.

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1805. See **Park, E. A.**

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1834. See **Osborne, T. B.**

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1928. [with **Jose F. Nonidez.**] Observations on the behavior of the nucleus and chromosomes in spermatocytes of Lasiopogon (Diptera).

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1840. See **Krumwiede, Charles.**

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1921. See **Larson, W. P.**

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1919. [with **Ross A. Gortner.**] A method for the estimation of the hydrophilic coloid content of expressed plant tissue fluids.

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1928. See **Metz, C. W.**

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1894. See **Chambers, Robert.**

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1885. [with **So Sabro Yamada.**] The effect of the administration of salvarsan in combination with various colloid substances on its toxicity.

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1903. [with **Paul E. Howe.**] Relation between the accumulation of globulins and the appearance of agglutinins in the blood of newborn calves.

**Orr, P. F.**

1755. See **Bronfenbrenner, J.**

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1834. [with **L. B. Mendel.**] Vitamine-A in oranges.

1876. [with **L. B. Mendel.**] Further observations on the occurrence of vitamine-B.

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1816. The effect of heat on the calcium salts and rennet.

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1851. See **Hess, A. F.**

**Park, E. A.**

1768. See **Shipley, P. G.**

1805. [with **G. F. Powers, P. G. Shipley, E. V. McCollum** and **Nina Simmonds.**] The prevention of rickets in the rat by means of radiation with the mercury vapor quartz lamp.

1807. See **McCollum, E. V.**

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1821. See **Eddy, Walter H.**

**Perme, Louis J.**

1836. See **Forward, D. D.**

**Pike, F. H.**

1829. See **Bowen, R. J.**

**Powers, G. F.**

1768. See **Shipley, P. G.**

1805. See **Park, E. A.**

**Presho, Elizabeth.**

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1884. See **Hanzlik, P. J.**

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1785. [with **Harry A. Cheplin.**] The therapeutic application of *Bacillus acidophilus.*

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1753. Identical twins in pigeons arise from ova of markedly aberrant size.

1869. An undescribed relation of the suprarenals to ovulation.

1931. See **Honeywell, H. E.**

**Ringer, A. I.**

1796. [with **H. Dubin** and **F. Hulton Frankel.**] The glycogen content of the tissues of diabetic animals and the influence of adrenalin thereon.

1797. Concerning antiketogenesis.

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1808. The effects of pituitary extract on the body temperature of animals rendered poikilothermous by destruction of the optic thalamus.

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1891. [with **Arthur W. Fuchs.**] Bio-radiological studies.

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1898. [with **Nathaniel Kleitman.**] The influence of sodium citrate on peristalsis.

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1749. Some plant sources of vitamines B and C.

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1814. On the weight increments of premature infants as compared with those of fetuses of the same gestation age and of those of full-term children.

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1748. See **Bronfenbrenner, J.**

1758. See **Bronfenbrenner, J.**

1881. See **Bronfenbrenner, J.**

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1770. A method for the preparation of cystin.

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1757. See **Weinberg, M. H.**

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1859. See **Galt, C. C.**
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1893. [with **James H. Crowdle.**] Detoxication in the organism of the fowl.
**Sherman, H. C.**
1786. [with **Marie Muhlfeld.**] Growth and reproduction upon simplified food supply. II. Influence of food upon mother and young during lactation period.
**Shelow, E.**
1821. See **Eddy, W. H.**
**Shipley, Paul G.**
1768. [with **E. A. Park, G. F. Powers, E. V. McCollum** and **Nina Simmonds.**] The prevention of the development of rickets in rats by sunlight.
1805. See **Park, E. A.**
1807. See **McCollum, E. V.**
1819. See **Park, E. A.**
**Simmonds, Nina.**
1768. See **Shipley, P. G.**
1805. See **Park, E. A.**
1807. See **McCollum, E. V.**
1819. See **Park, E. A.**
**Sisson, Warren R.**
1887. See **Talbot, Fritz.**
**Smith, A. H.**
1809. [with **Leah Ascham.**] The relation of splenectomy to growth and appetite in the rat.
**Smith, Clarence A.**
1759. [with **Olaf Bergeim** and **Philip B. Hawk.**] The antiscorbutic potency of strawberries.
**Snyder, L. H.**
1901. [with **W. J. Crozier.**] Selective pairing in gammarids.
**Sollmann, Torald.**
1945. [with **J. G. Brody.**] Influence of ischemia on infection.

**Stevens, Franklin A.**

1865. [with **Clifford Lamar.**] The effect of various proteins on streptolysin production.

**Swett, Madeleine.**

1863. See **Williams, J. R.**

**Spencer, Hope.**

1875. See **Woodruff, L. L.**

1908. See **Woodruff, L. L.**

**Stockard, C. R.**

1946. See **Papanicolaou, G. N.**

1947. See **Papanicolaou, G. N.**

**Trotter, Robert.**

1777. [with **Philip Edson** and **Robert Gesell.**] A comparison of the waves of blood pressure produced by slow and by rapid breathing.

**Talbot, Fritz.**

1887. [with **Warren R. Sisson.**] Basal metabolism in relation to body surface at different ages with special reference to prematurity.

**Ting, Gui Ching.**

1848. See **Macht, D. I.**

**Torrey, Harry Beal.**

1867. [with **Benjamin Horning.**] Hen feathering induced in the male fowl by feeding thyroid.

**Towne, E. B.**

1886. The so-called permanent polyuria of experimental diabetes insipidus.

**Ulrich, J. L.**

1833. See **Macht, D. I.**

**Unger, Lester J.**

1752. See **Hess, A. F.**

1804. See **Hess, A. F.**

1850. See **Hess, A. F.**

1851. See **Hess, A. F.**

**Van der Heyde, H. C.**

1762. See **Morse, Withrow.**

**Van Sant, Helen M.**

1882. See **Young, C. W.**

**Webster, Leslie T.**

1784. Experiments with *B. enteriditis* (*murium*) on normal and immune mice.

**Weinberg, Max H.**

1757. [with **T. Schubb.**] Experiment in new methods of therapy of paralysis agitans.

**Weiss, H.**

1880. See **Bronfenbrenner, J.**

**Weiss, Soma.**

1751. See **Hatcher, R. A.**

1935. [with **R. A. Hatcher.**] The emetic action of antimony and potassium tartrate (tartar emetic).

**Weller, Carl W.**

1812. Testicular changes in acute alcoholism in man and their relationship to blastophthoria.

**West, R. M.**

1922. See **Willamen, J. J.**

**Willamen, J. J.**

1922. [with **R. M. West.**] Correlations among the constituents of potato tubers.

**Williams, John R.**

1863. [with **Madeleine Swett.**] Hydrogen-ion concentration studies of solutions used for intravenous medication and clinical investigation.

**Wilson, D. C.**

1877. See **Hubbard, Roger S.**

**Winslow, C.-E. A.**

1888. [with **I. S. Falk.**] The mutual influence of acidity and salt concentration upon bacteria.

1889. [with **Margaret Hotchkiss.**] The influence of various salts upon bacterial growth.

**Woodruff, L. L.**

1875. [with **Hope Spencer.**] On the method of macronuclear dissolution during endomixus in paramecium aurelia.

1908. [with **Hope Spencer.**] Racial variations in *Blepharisma undulans.*

**Wright, Floyd R.**

1795. See **Hubbard, Roger S.**

**Yamada, So Sabro.**

1885. See **Oliver, Jean.**

**Young, Charles W.**

1882. [with **Helen M. Van Sant.**] The diagnosis of Kala-Azar by blood culture.

**Yudkin, A. M.**

1929. [with **R. A. Lambert.**] Location of the earliest changes in experimental xerophthalmia of rats.

1930. [with **R. A. Lambert.**] Lesions in the lacrimal glands of rats in experimental xerophthalmia.

**Zucker, T. F.**

1824. [with **A. M. Pappenheimer** and **Marion Barnett.**] Observations on cod-liver oil and rickets.

1825. [with **Margaret B. Gutman.**] The distribution of inorganic phosphate of the blood between plasma and cells.

# EXECUTIVE PROCEEDINGS.

## MAIN SOCIETY.

### One Hundred Seventeenth Meeting.

*Cornell University Medical College, October 19, 1921. President Wallace in the chair.*

*Members present:* Bailey, C. V., Binger, Bronfenbrenner, Calkins, Cecil, Chambers, Cohn, Curtis, Dochez, Dubin, Eddy, Edwards, Eggleston, Epstein, Fine, Funk, Greenwald, Hatcher, Hess, Jackson, H. C., Jobling, Kahn, Max, Kahn, M. H., Myers, Ottenberg, Pappenheimer, Park, E. A., Riddle, Ringer, Sherwin, Stark, Stevens, Swift, Teague, Thomas, Thro, Torrey, J. C., Wallace.

*Members elected:* Tadachika Minoura, Fritz Talbot.

### One Hundred Eighteenth Meeting.

*New York Post-Graduate Medical School, November 16, 1921. President Wallace in the chair.*

*Members present:* Baumann, E. J., Baumann, L., Barnett, Benedict, Cohen, Coleman, Crohn, Dubin, Edwards, Frankel, Jackson, H. C., Kleiner, Lynch, Myers, Ottenberg, Riddle, Ringer, Torrey, J. C., Wallace.

*Members elected:* Albert Fischer, Emil Goetsch, Clarence M. Jackson, W. B. Kirkham, Nicholas Kopeloff, J. Francis McClendon, Proviso V. Prewitt, Stanley P. Reimann, John P. Schneider, Harry Beal Torrey.

*Resignations:* William W. Ford.

### One Hundred Nineteenth Meeting.

*Rockefeller Institute for Medical Research, December 21, 1921. President Wallace in the chair.*

*Members present:* Austin, Avery, Bailey, C. V., Burton-Opitz, Cohen, M., Crohn, Eddy, Funk, Gates, Greenwald, Hastings, Hess, Jackson, H. C. Kopeloff, Levy, MacNeal, Myers, Pellini, Park, E. A., Salant, Sittenfield, Uhlenhuth, Van Slyke, D. D., Wallace, Zucker.

*Members elected:* S. Bayne-Jones, Guy W. Clark, George R. Cowgill, G. L. Foster, G. A. Friedman, Hubert Mann, Michael Ringer, Alfred Shohl.

### One Hundred Twentieth Meeting.

*College of Physicians and Surgeons, January 18, 1922. President Wallace in the chair.*

*Members present:* Bailey, C. V., Barr, Burton-Opitz, Cohn, A. E., Eddy, Edwards, Glaser, Greenwald, Friedman, Halsey, Hastings, Hess, Jobling, Kopeloff, Levy, Lieb, Little, Lynch, Mueller, Myers, Pappenheimer, Prewitt, Ringer, M., Scott, E. L., Scott, G. G., Sherwin, Stark, Stevens, Teague, Wallace, Williams, H. B., Zucker.

*Members elected:* Rudolph J. Anderson, Clyde Brooks, J. Howard Brown, Harry D. Clough, William H. Cole, Karl S. Lashley, Henry A. Mattill, Ralph R. Mellon, Chauncey John V. Pettibone, Andrew Theodore Rasmussen, Lucius L. Van Slyke.

*Resignations:* R. W. Hegner.

### One Hundred Twenty-first Meeting.

*University and Bellevue Hospital Medical College, February 15, 1922. President Wallace in the chair.*

*Members present:* Anderson, Draper, Eddy, Gettler, Jackson, H. C., Kopeloff, Levin, I., Maltaner, Mueller, Myers, MacNeal, Prewitt, Rose, A. R., Teague, Torrey, J. C., Wallace.

*Members elected:* S. A. Goldberg, George L. Hoffmann, L. A. Maynard, Fred T. Rogers, Robert W. Thatcher, W. S. Thomas.

*Honorary member elected:* William H. Welch.

*Resignations:* W. W. Cort, Otto Folin, J. B. Leathes, J. R. Mohler, Charles E. Simon, H. A. Spoehr, W. H. Taliaferro, J. V. Todd.

### One Hundred Twenty-second Meeting.

*Presbyterian Hospital, March 15, 1922. President Wallace in the chair.*

*Members present:* Bailey, C. V., Baumann, E. J., Binger, Brooks, S. C., Cole, R., Cowgill, Dochez, Eddy, Fischer, Friedman, G. A., Goldfarb, Harris, I., Hastings, Hess, Jackson, H. C., Jobling, Lundsgaard, Mackenzie, Myers, Pellini, Riddle, Stark, Stevens, Swift, Torrey, H. B., Wallace.

*Members elected:* Halsey J. Bagg, M. S. Dooley, P. G. Fish, Frank P. Knowlton, Charles Krumwiede, James M. Sherwin, John R. Williams.

The following changes in the constitution were presented and upon motion, were unanimously adopted.

## PRESENT CONSTITUTION.

### *Article V. Officials.*

*Section 1. Officers.*—The officers shall be a President, a Vice-President, and a Secretary-Treasurer.

They shall be elected from those members whose scientific work is conducted within the limits of Greater New York.

*Section 2. Council.*—The Council shall consist of the President, the Vice-President, the Secretary-Treasurer, and two members of the Society, one elected annually to serve two years. Ex-Presidents of the Society shall be permanent members of the Council.

*Section 3. Nomination and Election.—A.* Nominations of officers shall be made in the regular session immediately preceding the annual business meeting.

## *SUGGESTED AMENDMENTS.*

### *Article V. Officials.*

*Section 1. Officers.*—The elective officers shall be a President, a Vice-President, a Secretary-Treasurer and two Councilors.

They shall be elected from those members whose scientific work is conducted within the limits of Greater New York.

The Chairman of each local branch shall be *ex-officio* a Vice-President of the Society.

*Section 2. Council.*—The Council shall consist of the President, the Vice-Presidents, the Secretary-Treasurer and two Councilors, one of whom shall be elected annually to serve two years. Ex-Presidents of the Society shall be permanent members of the Council.

*Section 3. Nominations.—A.* At each annual meeting there shall be elected a Nominating Committee consisting of seven members from as many different scientific institutions, to serve for the ensuing year. For the term ending with the annual meeting in 1922, this committee shall be appointed by the President. For all ensuing terms, candidates for service on this committee shall be nominated by a primary vote of the whole Society and elected, by ballot, by the members in attendance at the annual business meetings. The Secretary of the Society shall act as the secretary of this committee.

*B.* Every member of the Society shall be eligible to membership in the Nominating Committee, but no one shall be eligible to serve more than two terms in succession.

*C.* It shall be the duty of the Nominating Committee to present to the Secretary of the Society, before the March meeting, at least one nomination for each of the elective offices.

*Section 4. Election.—A.* It shall be the duty of the Secretary to forward to all the members of the Society, with the preliminary announcements of the April meeting, an official ballot giving a list of all nominations made regularly by the Nominating Committee, with spaces for the insertion of independent votes for the officers and for the primary nominations for members in the new Nominating Committee. With the presentation of this list, the Secretary shall notify the members of the Society that each may participate in the election of all the officers and in the primary nomination of members of the Nominating Committee.

The Secretary shall present to the tellers, appointed by the President to take charge of the election, all signed ballots forwarded by absent members or received from members in attendance.

*B.* At each annual business meeting, immediately after the announcement of the returns of the primary nominations for membership in the Nominating Committee, the members present shall elect, from those thus formally put in primary nomination, the members of the new Nominating Committee, *at least four of whom shall be resident members.* The term of office shall be for the ensuing year.

*Article VIII. Local Branches.*

*Section 3. Representation on the Council.*—Each local branch shall elect a chairman who shall be an *ex-officio* member of the Council of the Society with the rank of Vice-President.

The following members of the Society were appointed by the President to act as a Nominating Committee to nominate officers for the ensuing year: Drs. Calkins, Cannon, Carlson, Lusk, Shaffer, Van Slyke, D. D. and Zinsser.

### One Hundred Twenty-third Meeting. (Nineteenth Annual Meeting.)

*College of the City of New York, April 19, 1922. President Wallace in the chair.*

*Members present:* Bailey, C. V., Baumann, E. J., Browne, W. W., Chambers, Churchman, DuBois, Eddy, Edwards, Famulener, Friedman, Hess, Hooper, Jackson, H. C., Jobling, MacNeal, Mann, H., Marine, Myers, Noble, Prewitt, Ringer, Rose, A. R., Ryan, Scott, G. A., Sherwin, Thro, Torrey, J. C., Wallace, Winslow.

*Members elected:* Ralph H. Boots, Moyer S. Fleisher, George Harrup, Jr., Albert Kuntz, August G. Pohlman, William D. Sansum, J. E. Thomas.

*Officers of the Society elected:* George B. Wallace, President; James W. Jobling, Vice-President, Holmes C. Jackson, Secretary-Treasurer; Member of the Council, Eugene F. DuBois.

*Nominating Committee for April, 1923, elected:* A. J. Carlson, Graham Lusk, W. J. MacNeal, L. B. Mendel, W. H. Park, D. D. Van Slyke, G. H. Whipple.

*Endowment Fund:* The Treasurer reported that through contributions made by members of the Society, the Meltzer Endowment Fund now amounts to $4108.50.

A request was granted for the founding of a Western New York branch of the Society.

The annual dinner was held at the College of the City of New York following the one hundred twenty-third meeting. The following members were present: Bailey, C. V., Barr, Baumann, E. J., Browne, W. W., Chambers, Dubin, DuBois, Eddy, Friedman, Funk, Famulener, Goldfarb, Hess, Hooper, Jackson, H. C., Jobling, MacNeal, Mann, H., Myers, Noble, Prewitt, Ringer, M.

Rose, A. R., Ryan, Scott, G. G., Sherwin, Thro, Torrey, J. C., Wallace, Winslow.

### One Hundred Twenty-fourth Meeting.

*Columbia University, May 17, 1922. Vice-President Jobling in the chair.*

*Members present:* Bagg, Coombs, Famulener, Jackson, H. C., Jobling, Lynch, Mann, H., Metz, Myers, Riddle, Rose, A. R., Scott, E. L., Senior, Stark, Zucker.

*Members elected:* Samuel Amberg, Mary E. Collett, William Croker, Max Shaw Dunn, I. S. Falk, Samuel R. Haythorn, Henry F. Helmholtz, Theophile Karl Kruse, William Swindler McEllory, Maud L. Menten, John Spangler Nicholas, Howard H. Permar, M. J. Schlesinger, Charles V. Taylor, George B. Wislocki.

*Resignations:* W. E. MacCallum.

## PACIFIC COAST BRANCH.

### Thirty-first Meeting.

*Stanford University, California, October 17, 1921.*

*Members present:* Alsberg, Alvarez, Barnett, Bloor, Cowan, Dickson, Evans, Faber, Gesell, Hall, Hanzlik, Hewlett, Hurwitz, Langstroth, Lucas, Manwaring, Martin, Ophüls, Schmidt, Smith, Swain, Taylor, Walker.

### Thirty-second Meeting.

*University of California Hospital, San Francisco, December 15, 1921.*

*Members present:* Addis, Alsberg, Alvarez, Barnett, Dickson, Fleischer, Hanzlik, Langstroth, Lucas, Oliver, Ophüls, Schmidt, Taylor, Walker.

### Thirty-third Meeting.

*Standford Medical School, San Francisco, February 15, 1922.*

*Members present:* Addis, Alvarez, Barnett, Beckwith, Blatherwick, Clark, Cowan, Dickson, Faber, Foster, Hanzlik, Hewlett, Kofoid, Lucas, Martin, Mehrtens, Oliver, Ophüls, Schmidt, Towne.

### Thirty-fourth Meeting.

*University of California, Berkeley, April 12, 1922.*

*Members present:* Alsberg, Bloor, Clark, Cowan, Dickson, Faber, Foster, Gesell, Hall, Lucas, Martin, Oliver, Ophüls, Schmidt, Walker.

## MINNESOTA BRANCH.

### First Meeting.

*University of Minnesota, Minneapolis, October 12, 1921.*

*Members present:* Bell, Brown, E. D., Eckles, Gortner, Hayes, Henrici, Hirschfelder, Jackson, C. M., Larson, Lund, Lyon, McClendon, Palmer, L. S., Scammon, Schlutz, Scott, F. H., Stakman, Willaman.

### Second Meeting.

*University of Minnesota, Minneapolis, December 14, 1921.*

*Members present:* Brown, Eckles, Fahr, Gortner, Henrici, Hirschfelder, Larson, Lund, Lycon, Palmer, Scammon, Scott, F. H.

### Third Meeting.

*University of Minnesota, Minneapolis, February 8, 1922.*

*Members present:* Brown, E. D., Eckles, Gortner, Henrici, Hirschfelder, Lund, McClendon, Pettibone, Schlutz, Scott, F. H.

### Fourth Meeting.

*University of Minnesota, Minneapolis, April 12, 1922.*

*Members present:* Brown, E. D., Eckles, Gortner, Henrici, Hirschfelder, Jackson, C. M., Lund, Mann, McClendon, Palmer, L. S., Rasmussen, Scammon, Schlutz, Willaman.

### Fifth Meeting.

*University of Minnesota, Minneapolis, May 10, 1922.*

*Members present:* Bell, Brown, E. D., Fitch, Henrici, Jackson, C. M., Kingsbury, Larson, Lyon, McClendon, Scammon, Rasmussen.

## WESTERN NEW YORK BRANCH.

### First Meeting. (Organization.)

*University of Buffalo, Buffalo, April 8, 1922.*

*Members present:* Anderson, Collins, Hubbard, Mattill, Mellon, Murlin, Simpson, Whipple, Williams, H. U.

### Second Meeting.

*Cornell University, Ithaca, May 20, 1922.*

*Members present:* Clough, Collins, Dooley, Fish, Goldberg, Hubbard, Knowlton, Mattill, Maynard, Mellon, Murlin, Simpson, Thomas.

# REGISTER OF NAMES AND INSTITUTIONAL CONNECTION OF THE MEMBERS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE.

## HONORARY MEMBERS.

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| Councilman, William T. | Harvard University |
| Reichert, Edward T. | University of Pennsylvania |
| Welch, William H. | Johns Hopkins University |

## ACTIVE MEMBERS.

| | |
|---|---|
| Abbott, Alexander C. | University of Pennsylvania |
| Abel, John J. | Johns Hopkins University |
| Adami, J. George | University of Liverpool, England |
| Addis, Thomas | Lane Hospital, San Francisco |
| Adler, Herman M. | Juvenile Psychopathic Institute, Chicago |
| Alexander, Harry L. | Cornell University Medical College, N. Y. City. |
| Allen, Bennet M. | University of Kansas |
| Alsberg, Carl L. | Leland Stanford University |
| Alvarez, Walter C. | University of California, Medical School |
| Amoss, Harold L. | Rockefeller Institute, N. Y. City |
| Anderson, John F. | Rutgers College |
| Anderson, Rudolph J. | N. Y. Agricultural Experiment Station |
| Atkinson, James P. | New York City Health Department |
| Auer, John | St. Louis University |
| Austin, J. Harold | University of Pennsylvania |
| Avery, O. T. | Rockefeller Institute, N. Y. City |
| | |
| Baehr, George | Mt. Sinai Hospital, N. Y. City |
| Bagg, Halsey J. | Memorial Hospital, N. Y. City |
| Bailey, C. H. | Columbia University |
| Bailey, Cameron V. | N. Y. Post-Graduate Medical School |
| Bailey, Harold C. | Cornell University Medical College, N. Y. City |
| Baitsell, George A. | Yale University |
| Balls, A. K. | Peekskill, New York |
| Banta, A. M. | Station for Exp. Evolution, Cold Spring Harbor, N. Y. |
| Banzhaf, Edwin J. | N. Y. Health Department |
| Barber, W. Howard | New York University |
| Barbour, Henry G. | McGill University |
| Bardeen, Charles R. | University of Wisconsin |
| Barnett, George D. | Leland Stanford University |
| Barr, David P. | Cornell University Medical College, N. Y. City |
| Baumann, E. J. | Montefiore Home, N. Y. City |
| Baumann, Louis | Presbyterian Hospital, N. Y. City |

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|---|---|
| Bayne-Jones, S. | Johns Hopkins University |
| Beckwith, T. D. | University of California |
| Bell, E. T. | University of Minnesota |
| Benedict, S. R. | Cornell University Medical College, N. Y. City |
| Berg, William N. | Bureau of Animal Industry, Washington, D. C. |
| Bergeim, Olaf | Jefferson Medical College |
| Bergey, David D. | University of Pennsylvania |
| Binger, Carl A. L. | Hospital of the Rockefeller Institute |
| Blakesley, Albert F. | Station for Exp. Evolution, Cold Spring Harbor, N. Y. |
| Blatherwick, Norman R. | Potter Metabolic Clinic, Santa Barbara |
| Bloor, W. R. | University of California |
| Boeck, William C. | University of Wyoming |
| Boots, Ralph H. | Rockefeller Institute, N. Y. City |
| Bronfenbrenner, J. | Harvard Medical School |
| Brooks, Clyde | University of Alabama |
| Brooks, Harlow | New York University |
| Brooks, S. C. | Hygienic Laboratory, Washington, D. C. |
| Brown, E. D. | University of Minnesota |
| Brown, J. Howard | Rockefeller Institute, Princeton, N. J. |
| Brown, Wade H. | Rockefeller Institute, N. Y. City |
| Browne, W. W. | College of the City of New York |
| Bull, C. G. | Johns Hopkins University |
| Bunting, C. H. | University of Wisconsin |
| Burnett, Theodore C. | University of California |
| Burrows, M. T. | Washington University Medical School |
| Burton-Opitz, Russell | Columbia University |

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| Calkins, Gary N. | Columbia University |
| Cannon, Walter B. | Harvard Medical School |
| Carlson, A. J. | University of Chicago |
| Carrel, Alexis | Rockefeller Institute, N. Y. City |
| Caulfeild, A. H. | University of Toronto, Canada |
| Cecil, R. L. | Bellevue Hospital, N. Y. City |
| Chace, Arthur F. | N. Y. Post-Graduate Medical School |
| Chambers, Robert | Cornell University Medical College, N. Y. City |
| Chidester, F. E. | University of West Virginia |
| Chittenden, R. H. | Yale University |
| Churchman, John W. | Cornell University Medical College, N. Y. City |
| Clark, Guy W. | University of California |
| Clark, P. F. | University of Wisconsin |
| Clowes, G. H. A. | Indianapolis, Indiana |
| Coca, A. F. | Cornell University Medical College, N. Y. City |
| Cohen, Barnett | U. S. Hygienic Laboratory, Washington, D. C. |
| Cohen, Martin | N. Y. Post-Graduate Medical School |
| Cohn, A. E. | Rockefeller Institute, N. Y. City |
| Cole, L. J. | University of Wisconsin |
| Cole, Rufus I. | Rockefeller Institute, N. Y. City |
| Cole, William H. | Lake Forest College, Lake Forest, Ill. |
| Coleman, Warren | New York University |
| Collins, Katharine R. | Division of Laboratories, Buffalo City Hospitals |

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| Conklin, E. G. | Princeton University |
| Cooke, J. V. | Washington University Medical School |
| Coombs, Helen C. | Columbia University |
| Corner, George V. | Johns Hopkins Medical School |
| Cowan, John F. | Stanford University Hospital, San Francisco |
| Crampton, C. Ward | Department of Education, New York City |
| Crile, George W. | Western Reserve University |
| Crohn, Burrill B. | Mt. Sinai Hospital, N. Y. City |
| Crozier, W. J. | Rutgers College |
| Cowgill, George R. | Yale University |
| Cullen, Glenn E. | University of Pennsylvania |
| Cunningham, R. S. | Johns Hopkins University |
| Curtis, Maynie R. | Columbia University |
| Cushing, Harvey W. | Harvard Medical School |

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| Dakin, H. D. | Ossining, N. Y. |
| Davenport, C. B. | Station for Exp. Evolution, Cold Spring Harbor, N. Y. |
| DeKruif, Paul H. | Rockefeller Institute, N. Y. City |
| Dickson, E. C. | Stanford University Medical School |
| Dochez, A. R. | Presbyterian Hospital, N. Y. City |
| Donaldson, H. H. | Wistar Institute, Philadelphia |
| Dooley, M. S. | Syracuse University |
| Draper, George W. | Columbia University |
| Draper, John W. | New York City |
| Dresbach, M. | Albany Medical College |
| Dubin, Harry E. | N. Y. City |
| DuBois, E. F. | Cornell University Medical College, N. Y. City |
| Duggar, B. M. | Missouri Botanical Garden |
| Dutcher, R. Adams | Pennsylvania State College |
| Duval, C. W. | Tulane University |

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| Eckles, C. H. | University of Minnesota |
| Eddy, Walter H. | Columbia University |
| Edmunds, C. W. | University of Michigan |
| Edwards, D. J. | Cornell University Medical College, N. Y. City |
| Eggleston, Cary | Cornell University Medical College, N. Y. City |
| Eggstein, Andrew | Columbia University |
| Eisenbrey, A. B. | Western Reserve University |
| Elsberg, Charles A. | Mt. Sinai Hospital, N. Y. City |
| Elser, W. J. | Cornell University Medical College, N. Y. City |
| Epstein, A. A. | Mt. Sinai Hospital, N. Y. City |
| Erdmann, Rhoda | Berlin, Germany |
| Erlanger, Joseph | Washington University Medical School |
| Evans, Herbert M. | University of California |
| Ewing, E. M. | Napoleonville, Miss. |
| Ewing, James | Cornell University Medical College, N. Y. City |
| Eyster, J. A. E. | University of Wisconsin |

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| Faber, Harold K. | Stanford Medical School, San Francisco |
| Fahr, George | University of Minnesota |

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| Falk, K. George | Roosevelt Hospital, N. Y. City |
| Famulener, L. W. | St. Luke's Hospital, N. Y. City |
| Field, Cyrus W. | N. Y. City |
| Fine, M. S. | Battle Creek, Mich. |
| Fischer, Albert | Rockefeller Institute, N. Y. City |
| Fischer, Martin H. | General Hospital, Cincinnati |
| Fish, Pierre A. | Cornell University |
| Fitch, C. P. | University of Minnesota, St. Paul |
| Fitzgerald, J. G. | University of Toronto |
| Fleischer, Moyer S. | St. Louis University |
| Fleischner, E. C. | University of California, San Francisco |
| Flexner, Simon | Rockefeller Institute, N. Y. City |
| Flournoy, Thomas | House of Mercy Hospital, Pittsfield, Mass. |
| Foster, Goodwin L. | University of California |
| Foster, Nellis B. | New York Hospital, N. Y. City |
| Frankel, Florence Hulton | New York University |
| Friedman, G. A. | Columbia University |
| Funk, Casimir | N. Y. City |

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|---|---|
| Gager, C. Stuart | Brooklyn Botanical Gardens |
| Gates, Frederick L. | Rockefeller Institute, N. Y. City |
| Gay, F. P. | University of California |
| Gaylord, H. R. | Gratwick Laboratory, Buffalo |
| Gesell, Robert A. | University of California |
| Gettler, A. O. | New York University |
| Gibson, R. B. | Iowa State University |
| Gies, William J. | Columbia University |
| Githens, T. S. | Philadelphia, Pa. |
| Givens, Maurice H. | Western Pennsylvania Hospital, Pittsburgh |
| Glaser, Otto | Amherst College |
| Goetsch, Emil | Long Island College Hospital, N. Y. City |
| Goldberg, S. A. | Cornell University |
| Goldfarb, A. J. | College of the City of New York |
| Goldschmidt, Samuel | University of Pennsylvania |
| Gortner, R. A. | University of Minnesota, St. Paul |
| Greenwald, Isidor | Roosevelt Hospital, N. Y. City |
| Gregory, Louise H. | Barnard College |
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| Guthrie, C. C. | University of Pittsburgh |

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|---|---|
| Hale, Worth | Harvard Medical School |
| Hall, Ivan C. | University of California |
| Halsey, Robert | N. Y. Post-Graduate Medical School |
| Halsted, W. S. | Johns Hopkins University |
| Hanzlik, P. J. | Stanford University, San Francisco |
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| Harrison, R. G. | Yale University |
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| Hartwell, John A. | Cornell University Medical College, N. Y. City |

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|---|---|
| Harvey, E. Newton | Princeton University |
| Hastings, A. Baird | Rockefeller Institute, N. Y. City |
| Hatai, Shinkishi | Tohoku Imperial University |
| Hatcher, R. A. | Cornell University Medical College, N. Y. City |
| Hawk, P. B. | Jefferson Medical College |
| Hayes, H. K. | University of Minnesota, St. Paul |
| Henderson, Lawrence J. | Harvard Medical School |
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| Hoffman, George L. | Western Pennsylvania Hospital, Pittsburgh, Pa. |
| Holman, W. L. | Stanford University |
| Holmes, S. J. | University of California |
| Hooker, Davenport | University of Pittsburgh |
| Hooper, Charles W. | Brooklyn, N. Y. |
| Hopkins, J. Gardner | Columbia University |
| Hoskins, R. G. | Ohio State University |
| Host, H. F. | Kristiania, Norway |
| Howe, Paul E. | Rockefeller Institute, N. Y. City |
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| Hubbard, Roger S. | Clifton Springs Sanitarium, N. Y. |
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| Hunt, Reid | Harvard Medical School |
| Hunter, Andrew | University of Toronto |
| Hurwitz, Samuel | University of California, San Francisco |

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| Jackson, C. M. | University of Minnesota |
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| Jackson, Holmes C. | New York University |
| Jacobs, Walter A. | Rockefeller Institute, N. Y. City |
| Janney, Nelson W. | Los Angeles, Cal. |
| Jennings, H. S. | Johns Hopkins University |
| Jobling, J. W. | Columbia University |
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| Joseph, Don R. | St. Louis University Medical School |

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| Kahn, Morris H. | Beth Israel Hospital, N. Y. City |
| Kahn, R. L. | Michigan Department of Health, Lansing |
| Karsner, H. T. | Lakeside Hospital, Cleveland |
| Kast, Ludwig | N. Y. Post-Graduate Medical School |
| Kellogg, V. L. | National Research Council, Washington, D. C. |
| Kendall, E. C. | University of Minnesota, Rochester |
| Killian, J. A. | N. Y. Post-Graduate Medical School |
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| Kinsella, Ralph A. | St. Louis University Medical School |

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| Knudson, Arthur | Albany Medical College, N. Y. |
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| Krumbhaar, E. B. | Philadelphia General Hospital, Philadelphia |
| Krumwiede, Charles | New York University |
| Kuntz, Albert | St. Louis University |

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| Mayer, A. G. | Carnegie Institute, Washington, D. C. |
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| Mosenthal, H. O. | N. Y. Post-Graduate Medical School |
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| Muller, Herman J. | University of Texas |
| Murlin, John R. | University of Rochester |
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| Scammon, R. E. | University of Minnesota |
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| Smith, Phillip E. | University of California |

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| Strong, Richard P. | Harvard Medical School |
| Strouse, Solomon | Northwestern University |
| Sturtevant, A. H. | Columbia University |
| Swain, R. E. | Leland Stanford University |
| Sweet, J. Edmund | University of Pennsylvania |
| Swift, H. F. | Rockefeller Institute, N. Y. City |
| Symmers, Douglas | New York University |

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| Talbot, Fritz B. | Harvard Medical School |
| Tashiro, Shiro | University of Cincinnati |
| Taylor, Alonzo E. | Stanford University |
| Taylor, R. M. | Maceo, Kentucky |
| Teague, Oscar | Columbia University |
| Ten-Broeck, Carl | Peking Union Medical College, China |
| Terry, B. T. | Vanderbilt School of Medicine |
| Thatcher, Robert W. | N. Y. Agric. Exp. Station, Geneva, N. Y. |
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| Thomas, J. E. | St. Louis University |
| Thomas, Walter S. | Clifton Springs Sanitarium, N. Y. |
| Thro, William C. | Cornell University Medical College, N. Y. City |
| Torrey, Harry B. | University of Oregon |
| Torrey, John C. | Cornell University Medical College, N. Y. City |
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| Tyzzer, E. E. | Harvard Medical School |

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| Underhill, Frank P. | Yale University |

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| Wallace, George B. | New York University |
| Warthin, Aldred S. | University of Michigan |
| Wasteneys, H. | University of Toronto, Canada |
| Watanabe, C. K. | Tokyo, Japan |

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| Welker, W. H. | University of Illinois |
| Weller, Carl V. | University of Michigan |
| West, C. J. | National Research Council, Washington, D. C. |
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| Willaman, J. J. | University of Minnesota, St. Paul |
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| Williams, Horatio B. | Columbia University |
| Williams, H. U. | University of Buffalo |
| Wilson, D. Wright | Johns Hopkins University |
| Wilson, Edmund B. | Columbia University |
| Winslow, C.-E. A. | Yale University |
| Wolbach, S. B. | Harvard University |
| Wolf, C. G. L. | Addenbrooke's Hospital, Cambridge, England |
| Wolstein, Martha | Rockefeller Institute, N. Y. City |
| Wood, Francis C. | Columbia University |
| Woodruff, L. L. | Yale University |
| Yatsu, Naohide | Keo University, Tokyo, Japan |
| Yerkes, Robert M. | Washington, D. C. |
| Zingher, Abraham | Department of Health, N. Y. City |
| Zinsser, Hans | Columbia University |
| Zucker, Theodore | Columbia University |

Total number of members at the close of the academic year, 1921–22:–492.

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1903–1922.

| | 1903–'04 | 1904–'05 | 1905–'06 | 1906–'07 | 1907–'08 | 1908–'09 |
|---|---|---|---|---|---|---|
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| Vice-President | Park | Ewing | Dunham | Dunham | Morgan | Morgan |
| Librarian | Lusk | Lusk | Lusk | —— | —— | —— |
| Treasurer | Calkins | Calkins | Calkins | Calkins | Calkins | Lusk |
| Secretary | Gies | Gies | Gies | Gies | Gies | Gies |

| | 1909–'10 | 1910–'10 | 1911–'12 | 1912–'13 | 1913–'14 | 1914–'15 |
|---|---|---|---|---|---|---|
| President | Lee | Morgan | Morgan | Ewing | Ewing | Lusk |
| Vice-President | Gies | Gies | Levene | Levene | Field | Gies |
| Treasurer | Lusk | Lusk | Lusk | Norris | Norris | Murlin |
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|---|---|---|---|---|---|---|
| President | Lusk | J. Loeb | Gies | Gies | Calkins | Calkins |
| Vice-President | Calkins | Gies | Auer | Auer | Wallace | Wallace |
| Sec'y-Treas. | Jackson | Jackson | Jackson | Jackson | Jackson | Jackson |
| Additional members of Council | Gies<br>Auer | Auer<br>DuBois | DuBois<br>Wallace | Wallace<br>Sherman | Sherman<br>Jobling | Jobling<br>Hess |

| | 1921–'22 | 1922–'23 |
|---|---|---|
| President | Wallace | Wallace |
| Vice-President | Jobling | Jobling |
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Edward T. Reichert, University of Pennsylvania.
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*New York City Departments. Health.*—James P. Atkinson, Edwin J. Banzhaf, R. L. Cecil, William H. Park, Anna W. Williams, A. Zingher. *Chief Medical Examiner.*—Charles Norris.

*New York Homoeopathic Medical College.*—I. S. Kleiner, Mary B. Stark.

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*Brooklyn Botanic Garden.*—C. Stuart Gager, O. E. White. *135 E. 34th St., N. Y. City.*—E. E. Butterfield. *17 E. 38th St., N. Y. City.*—J. W. Draper. *126 E. 64th St., N. Y. City.*—Cyrus W. Field. *141 W. 78th St., N. Y. City.*—A. I. Ringer.

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# PROCEEDINGS
OF THE
# SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

ONE HUNDRED SEVENTEENTH MEETING

CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK CITY
OCTOBER 19, 1921

AND

THIRTIETH MEETING

PACIFIC COAST BRANCH
BERKELEY, CALIFORNIA
OCTOBER 15, 1921

AND

FIRST MEETING

MINNESOTA BRANCH
MINNEAPOLIS, MINNESOTA
OCTOBER 12, 1921

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# EXPERIMENTAL BIOLOGY AND MEDICINE

ONE HUNDRED EIGHTEENTH MEETING

NEW YORK POST-GRADUATE MEDICAL
SCHOOL

NEW YORK CITY

NOVEMBER 16, 1921

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### Dates of the next two meetings:

December 21, 1921–January 18, 1922.

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OF THE

# SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE

ONE HUNDRED NINETEENTH MEETING

ROCKEFELLER INSTITUTE FOR MEDICAL RESEARCH
NEW YORK CITY
DECEMBER 21, 1921,

AND

SECOND MEETING

MINNESOTA BRANCH
MINNEAPOLIS, MINNESOTA
DECEMBER 14, 1921

VOLUME XIX

No. 3

NEW YORK
1921

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OF THE

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# EXPERIMENTAL BIOLOGY AND MEDICINE

ONE HUNDRED TWENTIETH MEETING

COLLEGE OF PHYSICIANS AND SURGEONS

NEW YORK CITY
JANUARY 18, 1922,

VOLUME XIX

No. 4

NEW YORK
1922

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# EXPERIMENTAL BIOLOGY AND MEDICINE

ONE HUNDRED TWENTY-FIRST MEETING

UNIVERSITY AND BELLEVUE HOSPITAL
MEDICAL COLLEGE

NEW YORK CITY
FEBRUARY 15, 1922

AND
THIRD MEETING
MINNESOTA BRANCH,
MINNEAPOLIS, MINNESOTA,
FEBRUARY 8, 1922

VOLUME XIX

No. 5

NEW YORK

1922

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NEW YORK CITY
MARCH 15, 1922

AND
THIRTY-SECOND MEETING
PACIFIC COAST BRANCH,
SAN FRANCISCO, CALIFORNIA
FEBRUARY 15, 1922

VOLUME XIX

No. 6

NEW YORK
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OF THE

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ONE HUNDRED TWENTY-THIRD MEETING

COLLEGE OF THE CITY OF NEW YORK

NEW YORK CITY
APRIL 19, 1922

AND

THIRTY-THIRD MEETING
PACIFIC COAST BRANCH,
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APRIL 12, 1922

AND

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VOLUME XIX

No. 7

NEW YORK

1922

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ONE HUNDRED TWENTY-FOURTH MEETING

COLUMBIA UNIVERSITY

NEW YORK CITY
MAY 17, 1922

AND

FIFTH MEETING
MINNESOTA BRANCH
MINNEAPOLIS, MINNESOTA
MAY 10, 1922

AND

SECOND MEETING
WESTERN NEW YORK BRANCH
ITHACA, NEW YORK
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VOLUME XIX
No. 8

NEW YORK
1922

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### Members elected at the one hundred twenty-fourth meeting:

Samuel Amberg, Mary E. Collett, William Croker, Max Shaw Dunn, I. S. Falk, Samuel R. Haythorn, Henry F. Helmholtz, Theophile Karl Kruse, William S. McEllory, Maud L. Menten, John S. Nicholas, Howard H. Permar, M. J. Schlesinger, Charles V. Taylor, George B. Wislocki.

### Dates of the next two meetings:

October 18, 1922–November 15, 1922.

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